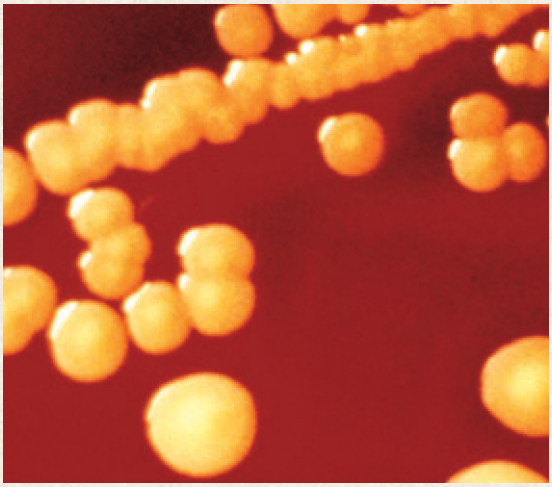
PART II

Microbial Nutrition, Growth, and Control

CHAPTER 5

Microbial Nutrition



Staphylococcus aureus forms large, golden colonies when growing on blood agar. This human pathogen causes diseases such as boils, abscesses, bacteremia, endocarditis, food poisoning, pharyngitis, and pneumonia.

Outline

Concepts

1. Microorganisms require about 10 elements in large quantities, in part because they are used to construct carbohydrates, lipids, proteins, and nucleic acids. Several other elements are needed in very small amounts and are parts of enzymes and cofactors.
2. All microorganisms can be placed in one of a few nutritional categories on the basis of their requirements for carbon, energy, and hydrogen atoms or electrons.
3. Nutrient molecules frequently cannot cross selectively permeable plasma membranes through passive diffusion. They must be transported by one of three major mechanisms involving the use of membrane carrier proteins. Eucaryotic microorganisms also employ endocytosis for nutrient uptake.
4. Culture media are needed to grow microorganisms in the laboratory and to carry out specialized procedures like microbial identification, water and food analysis, and the isolation of particular microorganisms. Many different media are available for these and other purposes.
5. Pure cultures can be obtained through the use of spread plates, streak plates, or pour plates and are required for the careful study of an individual microbial species.

The whole of nature, as has been said, is a conjugation of the verb to eat, in the active and passive.

—William Ralph Inge

To obtain energy and construct new cellular components, organisms must have a supply of raw materials or nutrients. **Nutrients** are substances used in biosynthesis and energy production and therefore are required for microbial growth. This chapter describes the nutritional requirements of microorganisms, how nutrients are acquired, and the cultivation of microorganisms.

Environmental factors such as temperature, oxygen levels, and the osmotic concentration of the medium are critical in the successful cultivation of microorganisms. These topics are discussed in chapter 6 after an introduction to microbial growth.

5.1 The Common Nutrient Requirements

Analysis of microbial cell composition shows that over 95% of cell dry weight is made up of a few major elements: carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. These are called **macroelements** or macronutrients because they are required by microorganisms in relatively large amounts. The first six (C, O, H, N, S, and P) are components of carbohydrates, lipids, proteins, and nucleic acids. The remaining four macroelements exist in the cell as cations and play a variety of roles. For example, potassium (K^+) is required for activity by a number of enzymes, including some of those involved in protein synthesis. Calcium (Ca^{2+}), among other functions, contributes to the heat resistance of bacterial endospores. Magnesium (Mg^{2+}) serves as a cofactor for many enzymes, complexes with ATP, and stabilizes ribosomes and cell membranes. Iron (Fe^{2+} and Fe^{3+}) is a part of cytochromes and a cofactor for enzymes and electron-carrying proteins.

All organisms, including microorganisms, require several **micronutrients** or **trace elements** besides macroelements. The micronutrients—manganese, zinc, cobalt, molybdenum, nickel, and copper—are needed by most cells. However, cells require such small amounts that contaminants in water, glassware, and regular media components often are adequate for growth. Therefore it is very difficult to demonstrate a micronutrient requirement. In nature, micronutrients are ubiquitous and probably do not usually limit growth. Micronutrients are normally a part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure. For example, zinc (Zn^{2+}) is present at the active site of some enzymes but is also involved in the association of regulatory and catalytic subunits in *E. coli* aspartate carbamoyltransferase (*see section 8.9*). Manganese (Mn^{2+}) aids many enzymes catalyzing the transfer of phosphate groups. Molybdenum (Mo^{2+}) is required for nitrogen fixation, and cobalt (Co^{2+}) is a component of vitamin B_{12}. Electron carriers and enzymes (pp. 157–64)

Besides the common macroelements and trace elements, microorganisms may have particular requirements that reflect the special nature of their morphology or environment. Diatoms (*see figure 26.6*c,d) need silicic acid (H_4SiO_4) to construct their beautiful cell walls of silica [$(SiO_2)_n$]. Although most bacteria do not require large amounts of sodium, many bacteria growing in saline lakes and oceans (*see pp. 123, 461*) depend on the presence of high concentrations of sodium ion (Na^+).

Finally, it must be emphasized that microorganisms require a balanced mixture of nutrients. If an essential nutrient is in short supply, microbial growth will be limited regardless of the concentrations of other nutrients.

5.2 Requirements for Carbon, Hydrogen, and Oxygen

The requirements for carbon, hydrogen, and oxygen often are satisfied together. Carbon is needed for the skeleton or backbone of all organic molecules, and molecules serving as carbon sources normally also contribute both oxygen and hydrogen atoms. They are the source of all three elements. Because these organic nutrients are almost always reduced and have electrons that they can donate to other molecules, they also can serve as energy sources. Indeed, the more reduced organic molecules are, the higher their energy content (e.g., lipids have a higher energy content than carbohydrates). This is because, as we shall see later, electron transfers release energy when the electrons move from reduced donors with more negative reduction potentials to oxidized electron acceptors with more positive potentials. Thus carbon sources frequently also serve as energy sources, although they don't have to. Oxidation-reduction reactions and energy (pp. 157–59)

One important carbon source that does not supply hydrogen or energy is carbon dioxide (CO_2). This is because CO_2 is oxidized and lacks hydrogen. Probably all microorganisms can fix CO_2—that is, reduce it and incorporate it into organic molecules. However, by definition, only **autotrophs** can use CO_2 as their sole or principal source of carbon. Many microorganisms are autotrophic, and most of these carry out photosynthesis and use light as their energy source. Some autotrophs oxidize inorganic molecules and derive energy from electron transfers. Photosynthetic carbon dioxide fixation (pp. 207–8)

The reduction of CO_2 is a very energy-expensive process. Thus many microorganisms cannot use CO_2 as their sole carbon source but must rely on the presence of more reduced, complex molecules such as glucose for a supply of carbon. Organisms that use reduced, preformed organic molecules as carbon sources are **heterotrophs** (these preformed molecules normally come from other organisms). As mentioned previously, most heterotrophs use reduced organic compounds as sources of both carbon and energy. For example, the glycolytic pathway produces carbon skeletons for use in biosynthesis and also releases energy as ATP and NADH. The glycolytic pathway (pp. 176–77)

A most remarkable nutritional characteristic of microorganisms is their extraordinary flexibility with respect to carbon sources. Laboratory experiments indicate that there is no natu-

rally occurring organic molecule that cannot be used by some microorganism. Actinomycetes will degrade amyl alcohol, paraffin, and even rubber. Some bacteria seem able to employ almost anything as a carbon source; for example, *Burkholderia cepacia* can use over 100 different carbon compounds. In contrast to these bacterial omnivores, some bacteria are exceedingly fastidious and catabolize only a few carbon compounds. Cultures of methylotrophic bacteria metabolize methane, methanol, carbon monoxide, formic acid, and related one-carbon molecules. Parasitic members of the genus *Leptospira* use only long-chain fatty acids as their major source of carbon and energy.

It appears that in natural environments complex populations of microorganisms often will metabolize even relatively indigestible human-made substances such as pesticides. Indigestible molecules sometimes are oxidized and degraded in the presence of a growth-promoting nutrient that is metabolized at the same time, a process called cometabolism. The products of this breakdown process can then be used as nutrients by other microorganisms. Degradation and microorganisms (pp. 1010–14)

5.3 Nutritional Types of Microorganisms

In addition to the need for carbon, hydrogen, and oxygen, all organisms require sources of energy and electrons for growth to take place. Microorganisms can be grouped into nutritional classes based on how they satisfy all these requirements (**table 5.1**). We have already seen that microorganisms can be classified as either heterotrophs or autotrophs with respect to their preferred source of carbon. There are only two sources of energy available to organisms: (1) light energy, and (2) the energy derived from oxidizing organic or inorganic molecules. **Phototrophs** use light as their energy source; **chemotrophs** obtain energy from the oxidation of chemical compounds (either organic or inorganic). Microorganisms also have only two sources for electrons. **Lithotrophs** (i.e., "rock-eaters") use reduced inorganic substances as their electron source, whereas **organotrophs** extract electrons from organic compounds. Photosynthesis light reactions (pp. 195–201); Oxidation of organic and inorganic molecules (pp. 176–95)

Despite the great metabolic diversity seen in microorganisms, most may be placed in one of four nutritional classes based on their primary sources of carbon, energy, and electrons (**table 5.2**). The large majority of microorganisms thus far studied are either photolithotrophic autotrophs or chemoorganotrophic heterotrophs. **Photolithotrophic autotrophs** (often called **photoautotrophs** or photolithoautotrophs) use light energy and have CO_2 as their carbon source. Eucaryotic algae and cyanobacteria employ water as the electron donor and release oxygen. Purple and green sulfur

Table 5.1 Sources of Carbon, Energy, and Electrons

Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source (*pp. 207–8*)[a]
Heterotrophs	Reduced, preformed, organic molecules from other organisms (*chapters 9 and 10*)
Energy Sources	
Phototrophs	Light (*pp. 195–201*)
Chemotrophs	Oxidation of organic or inorganic compounds (*chapter 9*)
Electron Sources	
Lithotrophs	Reduced inorganic molecules (*pp. 193–94*)
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.

bacteria cannot oxidize water but extract electrons from inorganic donors like hydrogen, hydrogen sulfide, and elemental sulfur. **Chemoorganotrophic heterotrophs** (often called **chemoheterotrophs,** chemoorganoheterotrophs, or even heterotrophs) use organic compounds as sources of energy, hydrogen, electrons, and carbon. Frequently the same organic nutrient will satisfy all these requirements. It should be noted that essentially all pathogenic microorganisms are chemoheterotrophs.

The other two nutritional classes have fewer microorganisms but often are very important ecologically. Some purple and green bacteria are photosynthetic and use organic matter as their electron donor and carbon source. These **photoorganotrophic heterotrophs** (photoorganoheterotrophs) are common inhabitants of polluted lakes and streams. Some of these bacteria also can grow as photoautotrophs with molecular hydrogen as an electron donor. The fourth group, the **chemolithotrophic autotrophs** (chemolithoautotrophs), oxidizes reduced inorganic compounds such as iron, nitrogen, or sulfur molecules to derive both energy and electrons for biosynthesis. Carbon dioxide is the carbon source. A few chemolithotrophs can derive their carbon from organic sources and thus are heterotrophic. Chemolithotrophs contribute greatly to the chemical transformations of elements (e.g., the conversion of ammonia to nitrate or sulfur to sulfate) that continually occur in the ecosystem. Photosynthetic and chemolithotrophic bacteria (sections 21.3, 22.1, and 22.3)

Although a particular species usually belongs in only one of the four nutritional classes, some show great metabolic flexibility and alter their metabolic patterns in response to environmental changes. For example, many purple nonsulfur bacteria (*see section 22.1*) act as photoorganotrophic heterotrophs in the absence of oxygen but oxidize organic molecules and function chemotrophically at normal oxygen levels. When oxygen is low, photosynthesis and oxidative metabolism may function simultaneously. Another example is provided by bacteria such as *Beggiatoa* (*see p. 501*) that rely on inorganic energy sources and organic (or sometimes CO_2) carbon sources. These microbes are sometimes called **mixotrophic** because they combine chemolithoautotrophic and heterotrophic metabolic processes. This sort of flexibility seems complex and confusing, yet it gives its possessor a definite advantage if environmental conditions frequently change.

1. What are nutrients, and on what basis are they divided into macroelements and micronutrients or trace elements? Describe some ways in which macroelements and micronutrients are used by an organism.
2. Define autotroph and heterotroph.
3. Discuss the ways in which microorganisms are classified based on their requirements for energy and electrons.
4. Describe the nutritional requirements of the four major nutritional groups and give some microbial examples of each. What is a mixotroph?

5.4 Requirements for Nitrogen, Phosphorus, and Sulfur

To grow, a microorganism must be able to incorporate large quantities of nitrogen, phosphorus, and sulfur. Although these elements may be acquired from the same nutrients that supply carbon, microorganisms usually employ inorganic sources as well. Biochemical mechanisms for the incorporation of nitrogen, phosphorus, and sulfur (pp. 210–14)

Nitrogen is needed for the synthesis of amino acids, purines, pyrimidines, some carbohydrates and lipids, enzyme cofactors, and other substances. Many microorganisms can use the nitrogen in amino acids, and ammonia often is directly incorporated through the action of such enzymes as glutamate dehydrogenase or glutamine synthetase and glutamate synthase (*see section 10.4*). Most phototrophs and many nonphotosynthetic microorganisms reduce nitrate to ammonia and incorporate the ammonia in assimilatory nitrate reduction (*see pp. 210–11*). A variety of bacteria (e.g., many cyanobacteria and the symbiotic bacterium *Rhizobium*) can reduce and assimilate atmospheric nitrogen using the nitrogenase system (*see section 10.4*).

Phosphorus is present in nucleic acids, phospholipids, nucleotides like ATP, several cofactors, some proteins, and other cell components. Almost all microorganisms use inorganic phosphate as their phosphorus source and incorporate it directly. Low phosphate levels actually limit microbial growth in many aquatic environments. Phosphate uptake by *E. coli* has been intensively studied. This bacterium can use both organic and inorganic phosphate. Some organophosphates such as hexose 6-phosphates can be taken up directly by transport proteins. Other organophosphates are often hydrolyzed in the periplasm by the enzyme alkaline phosphatase to produce inorganic phosphate, which then is transported across the plasma membrane. When inorganic phosphate is outside the bacterium, it crosses the outer membrane by the use of a porin protein channel. One of two transport systems subsequently moves the phosphate across the plasma membrane. At high phosphate concentrations, transport probably is due to the Pit system. When phosphate concentrations are low, the PST, (*p*hosphate-*s*pecific *t*ransport) system is more important. The PST system has higher affinity for phosphate; it is an ABC transporter (see pp. 101–2) and uses a periplasmic binding protein.

Sulfur is needed for the synthesis of substances like the amino acids cysteine and methionine, some carbohydrates, biotin, and thiamine. Most microorganisms use sulfate as a source of sulfur and reduce it by assimilatory sulfate reduction (*see section 10.4*); a few require a reduced form of sulfur such as cysteine.

5.5 Growth Factors

Microorganisms often grow and reproduce when minerals and sources of energy, carbon, nitrogen, phosphorus, and sulfur are supplied. These organisms have the enzymes and pathways nec-

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).

essary to synthesize all cell components required for their well-being. Many microorganisms, on the other hand, lack one or more essential enzymes. Therefore they cannot manufacture all indispensable constituents but must obtain them or their precursors from the environment. Organic compounds required because they are essential cell components or precursors of such components and cannot be synthesized by the organism are called **growth factors.** There are three major classes of growth factors: (1) amino acids, (2) purines and pyrimidines, and (3) vitamins. Amino acids are needed for protein synthesis, purines and pyrimidines for nucleic acid synthesis. **Vitamins** are small organic molecules that usually make up all or part of enzyme cofactors (*see section 8.6*), and only very small amounts sustain growth. The functions of selected vitamins, and examples of microorganisms requiring them, are given in **table 5.3.** Some microorganisms require many vitamins; for example, *Enterococcus faecalis* needs eight different vitamins for growth. Other growth factors are also seen; heme (from hemoglobin or cytochromes) is required by *Haemophilus influenzae,* and some mycoplasmas need cholesterol.

Knowledge of the specific growth factor requirements of many microorganisms makes possible quantitative growth-response assays for a variety of substances. For example, species from the bacterial genera *Lactobacillus* and *Streptococcus* can be used in microbiological assays of most vitamins and amino acids. The appropriate bacterium is grown in a series of culture vessels, each containing medium with an excess amount of all required components except the growth factor to be assayed. A different amount of growth factor is added to each vessel. The standard curve is prepared by plotting the growth factor quantity or concentration against the total extent of bacterial growth. Ideally the amount of growth resulting is directly proportional to the quantity of growth factor present; if the growth factor concentration doubles, the final extent of bacterial growth doubles. The quantity of the growth factor in a test sample is determined by comparing the extent of growth caused by the unknown sample with that resulting from the standards. Microbiological assays are specific, sensitive, and simple. They still are used in the assay of substances like vitamin B_{12} and biotin, despite advances in chemical assay techniques.

The observation that many microorganisms can synthesize large quantities of vitamins has led to their use in industry. Several water-soluble and fat-soluble vitamins are produced partly or completely using industrial fermentations. Good examples of such vitamins and the microorganisms that synthesize them are riboflavin (*Clostridium, Candida, Ashbya, Eremothecium*), coenzyme A (*Brevibacterium*), vitamin B_{12} (*Streptomyces, Propionibacterium,*

Pseudomonas), vitamin C (*Gluconobacter, Erwinia, Corynebacterium*), β-carotene (*Dunaliella*), and vitamin D (*Saccharomyces*). Current research focuses on improving yields and finding microorganisms that can produce large quantities of other vitamins.

1. Briefly summarize the ways in which microorganisms obtain nitrogen, phosphorus, and sulfur from their environment.
2. What are growth factors? What are vitamins? How can microorganisms be used to determine the quantity of a specific substance in a sample?

5.6 Uptake of Nutrients by the Cell

The first step in nutrient use is uptake of the required nutrients by the microbial cell. Uptake mechanisms must be specific—that is, the necessary substances, and not others, must be acquired. It does a cell no good to take in a substance that it cannot use. Since microorganisms often live in nutrient-poor habitats, they must be able to transport nutrients from dilute solutions into the cell against a concentration gradient. Finally, nutrient molecules must pass through a selectively permeable plasma membrane that will not permit the free passage of most substances. In view of the enormous variety of nutrients and the complexity of the task, it is not surprising that microorganisms make use of several different transport mechanisms. The most important of these are facilitated diffusion, active transport, and group translocation. Eucaryotic microorganisms do not appear to employ group translocation but take up nutrients by the process of endocytosis (*see section 4.5*). Plasma membrane structure and properties (pp. 46–48)

Facilitated Diffusion

A few substances, such as glycerol, can cross the plasma membrane by **passive diffusion.** Passive diffusion, often simply called diffusion, is the process in which molecules move from a region of higher concentration to one of lower concentration because of random thermal agitation. The rate of passive diffusion is dependent on the size of the concentration gradient between a cell's exterior and its interior (**figure 5.1**). A fairly large concentration gradient is required for adequate nutrient uptake by passive diffusion (i.e., the external nutrient concentration must be high), and the rate of uptake decreases as more nutrient is acquired unless it is used immediately. Very small molecules such as H_2O, O_2, and CO_2 often move across membranes by passive diffusion. Larger molecules, ions, and polar substances do not cross membranes by passive or simple diffusion.

The rate of diffusion across selectively permeable membranes is greatly increased by using carrier proteins, sometimes called **permeases,** which are embedded in the plasma membrane. Because a carrier aids the diffusion process, it is called **facilitated diffusion.** The rate of facilitated diffusion increases with the concentration gradient much more rapidly and at lower concentrations of the diffusing molecule than that of passive diffusion (figure 5.1). Note that the diffusion rate levels off or reaches a plateau above a specific gradient value because the carrier is saturated—that is, the carrier protein is binding and transporting as many solute molecules as possible. The resulting curve resembles an enzyme-substrate curve (*see section 8.6*) and is different from the linear response seen with passive diffusion. Carrier proteins also resemble enzymes in their specificity for the substance to be transported; each carrier is selective and will transport only closely related solutes. Although a carrier protein is involved, facilitated diffusion is truly diffusion. A concentration gradient spanning the membrane drives the movement of molecules, and no metabolic energy input is required. If the concentration gradient disappears, net inward movement ceases. The gradient can be maintained by transforming the transported nutrient to another compound or by moving it to another membranous compartment in eucaryotes. Interestingly, some of these carriers are related to the major intrinsic protein of mammalian eye lenses and thus belong to the MIP family of proteins. The two most widespread MIP channels in bacteria are aquaporins that transport water and glycerol facilitators, which aid glycerol diffusion.

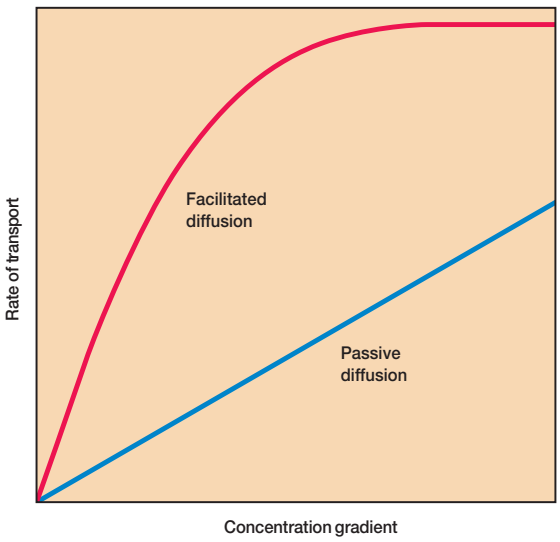


Figure 5.1 Passive and Facilitated Diffusion. The dependence of diffusion rate on the size of the solute's concentration gradient. Note the saturation effect or plateau above a specific gradient value when a facilitated diffusion carrier is operating. This saturation effect is seen whenever a carrier protein is involved in transport.

Although much work has been done on the mechanism of facilitated diffusion, the process is not yet understood completely. It appears that the carrier protein complex spans the membrane (**figure 5.2**). After the solute molecule binds to the outside, the carrier may change conformation and release the molecule on the cell interior. The carrier would subsequently change back to its original shape and be ready to pick up another molecule. The net effect is that a lipid-insoluble molecule can enter the cell in response to its concentration gradient. Remember that the mecha-

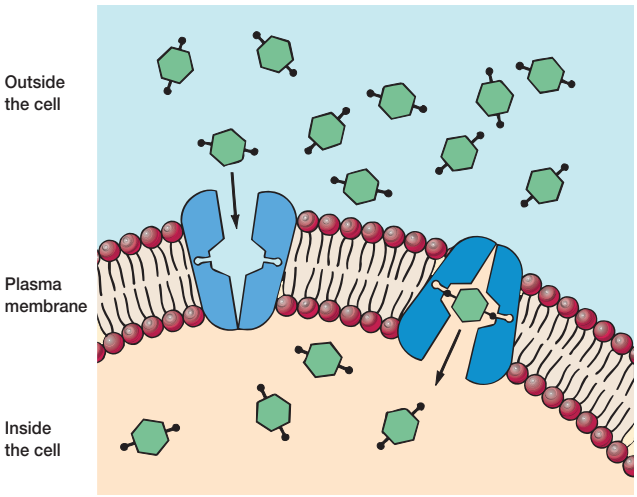


Figure 5.2 A Model of Facilitated Diffusion. The membrane carrier can change conformation after binding an external molecule and subsequently release the molecule on the cell interior. It then returns to the outward oriented position and is ready to bind another solute molecule. Because there is no energy input, molecules will continue to enter only as long as their concentration is greater on the outside.

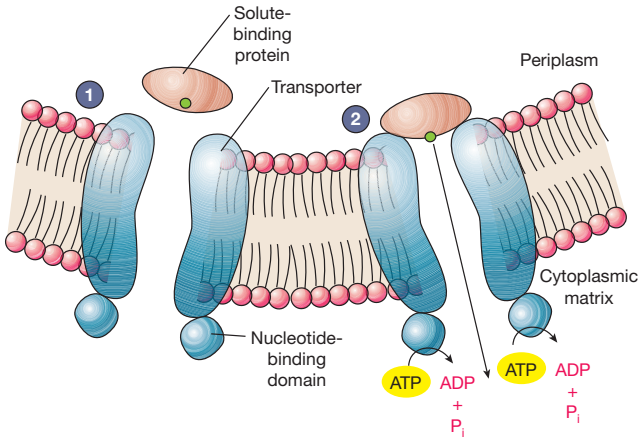


Figure 5.3 ABC Transporter Function. (*1*) The solute binding protein binds the substrate to be transported and approaches the ABC transporter complex. (*2*) The solute binding protein attaches to the transporter and releases the substrate, which is moved across the membrane with the aid of ATP hydrolysis. See text for details.

nism is driven by concentration gradients and therefore is reversible. If the solute's concentration is greater inside the cell, it will move outward. Because the cell metabolizes nutrients upon entry, influx is favored.

Facilitated diffusion does not seem to be important in procaryotes because nutrient concentrations often are lower outside the cell so that facilitated diffusion cannot be used in uptake. Glycerol is transported by facilitated diffusion in *E. coli, Salmonella typhimurium, Pseudomonas, Bacillus,* and many other bacteria. The process is much more prominent in eucaryotic cells where it is used to transport a variety of sugars and amino acids.

Active Transport

Although facilitated diffusion carriers can efficiently move molecules to the interior when the solute concentration is higher on the outside of the cell, they cannot take up solutes that are already more concentrated within the cell (i.e., against a concentration gradient). Microorganisms often live in habitats characterized by very dilute nutrient sources, and, to flourish, they must be able to transport and concentrate these nutrients. Thus facilitated diffusion mechanisms are not always adequate, and other approaches must be used. The two most important transport processes in such situations are active transport and group translocation, both energy-dependent processes.

Active transport is the transport of solute molecules to higher concentrations, or against a concentration gradient, with the use of metabolic energy input. Because active transport involves protein carrier activity, it resembles facilitated diffusion in some ways. The carrier proteins or permeases bind particular solutes with great specificity for the molecules transported. Similar solute molecules can compete for the same carrier protein in both facilitated diffusion and active transport. Active transport is also characterized by the carrier saturation effect at high solute concentrations (figure 5.1). Nevertheless, active transport differs from facilitated diffusion in its use of metabolic energy and in its ability to concentrate substances. Metabolic inhibitors that block energy production will inhibit active transport but will not affect facilitated diffusion (at least for a short time).

Binding protein transport systems or ***A*TP-*b*inding *c*assette transporters (ABC transporters)** are active in bacteria, archaea, and eucaryotes. Usually these transporters consist of two hydrophobic membrane-spanning domains associated on their cytoplasmic surfaces with two nucleotide-binding domains (**figure 5.3**). The membrane-spanning domains form a pore in the membrane and the nucleotide-binding domains bind and hydrolyze ATP to drive uptake. ABC transporters employ special substrate binding proteins, which are located in the periplasmic space of gram-negative bacteria (*see figure 3.23*) or are attached to membrane lipids on the external face of the gram-positive plasma membrane. These binding proteins, which also may participate in chemotaxis (*see pp. 66–68*), bind the molecule to be transported and then interact with the membrane transport proteins to move the solute molecule inside the cell. *E. coli* transports a variety of sugars (arabinose, maltose, galactose, ribose) and amino acids (glutamate, histidine, leucine) by this mechanism.

Substances entering gram-negative bacteria must pass through the outer membrane before ABC transporters and other

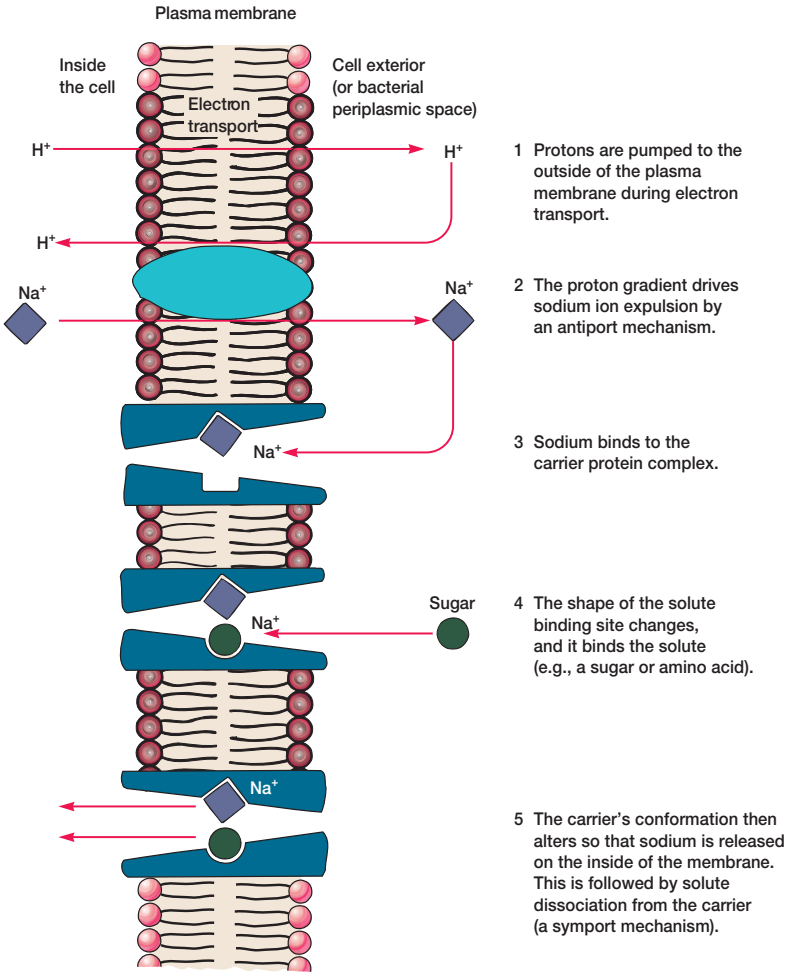


Figure 5.4 Active Transport Using Proton and Sodium Gradients. (*1*) Protons are pumped to the outside of the plasma membrane during electron transport. (*2*) The proton gradient drives sodium ion expulsion by an antiport mechanism. (*3*) Sodium binds to the carrier protein complex. (*4*) The shape of the solute binding site changes, and it binds the solute (e.g., a sugar or amino acid). (*5*) The carrier's conformation then alters so that sodium is released on the inside of the membrane. This is followed by solute dissociation from the carrier (a symport mechanism).

active transport systems can take action. There are several ways in which this is accomplished. When the substance is small, a generalized porin protein (*see p. 60*) such as OmpF can be used; larger molecules require specialized porins. In some cases (e.g., for uptake of iron and vitamin B_{12}), specialized high-affinity outer membrane receptors and transporters are used.

It should be noted that eucaryotic ABC transporters are sometimes of great medical importance. Some tumor cells pump drugs out using these transporters. Cystic fibrosis results from a mutation that inactivates an ABC transporter that acts as a chloride ion channel in the lungs.

Bacteria also use proton gradients generated during electron transport to drive active transport. The membrane transport proteins responsible for this process lack special periplasmic solute-binding proteins. The lactose permease of *E. coli* is a well-studied example. The permease is a single protein having a molecular weight of about 30,000. It transports a lactose molecule inward as a proton simultaneously enters the cell (a higher concentration of protons is maintained outside the membrane by electron transport chain activity). Such linked transport of two substances in the same direction is called **symport.** Here, energy stored as a proton gradient drives solute transport. Although the mechanism of transport is not completely understood, it is thought that binding of a proton to the transport protein changes its shape and affinity for the solute to be transported. *E. coli* also uses proton symport to take up amino acids and organic acids like succinate and malate. The chemiosmotic hypothesis (p. 187)

A proton gradient also can power active transport indirectly, often through the formation of a sodium ion gradient. For example, an *E. coli* sodium transport system pumps sodium outward in response to the inward movement of protons (**figure 5.4**). Such linked transport in which the transported substances move in opposite directions is termed **antiport.** The sodium gradient generated by this proton antiport system then drives the uptake of sugars and amino acids. A sodium ion could attach to a carrier protein, causing it to change shape. The carrier would then bind the sugar or amino acid tightly and orient its binding sites toward the cell interior. Because of the low intracellular sodium concentration, the sodium ion would dissociate from the carrier, and the other molecule would follow. *E. coli* transport proteins carry the sugar melibiose and the amino acid glutamate when sodium simultaneously moves inward.

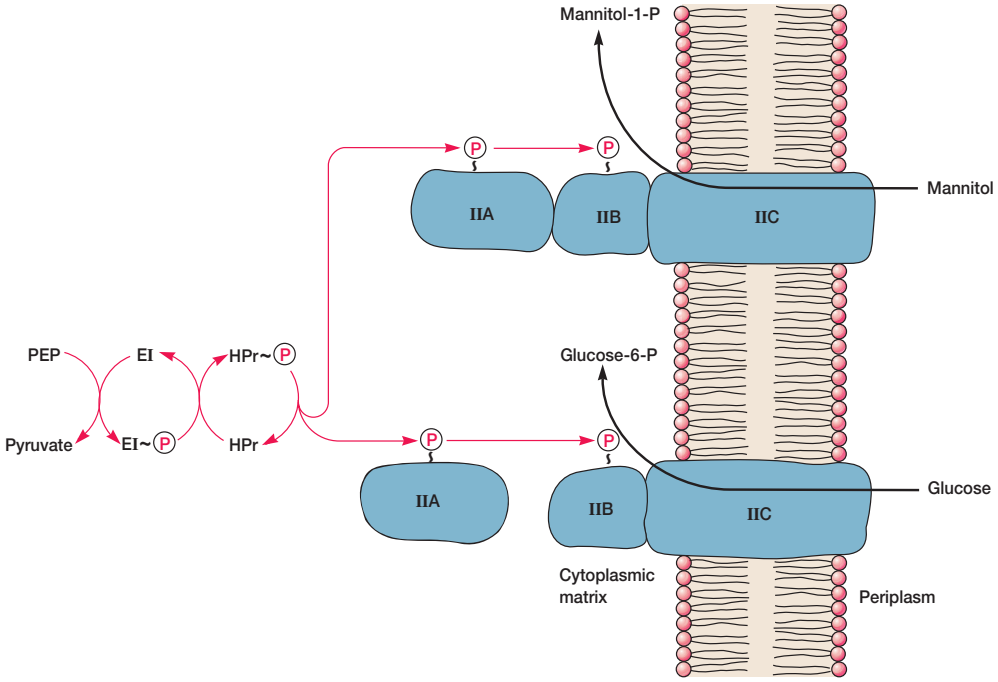


Figure 5.5 Group Translocation: Bacterial PTS Transport. Two examples of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) are illustrated. The following components are involved in the system: phosphoenolpyruvate (PEP), enzyme I (EI), the low molecular weight heat-stable protein (HPr), and enzyme II (EII). The high-energy phosphate is transferred from HPr to the soluble EIIA. EIIA is attached to EIIB in the mannitol transport system and is separate from EIIB in the glucose system. In either case the phosphate moves from EIIA to EIIB, and then is transferred to the sugar during transport through the membrane. Other relationships between the EII components are possible. For example, IIA and IIB may form a soluble protein separate from the membrane complex; the phosphate still moves from IIA to IIB and then to the membrane domain(s).

Sodium symport or cotransport also is an important process in eucaryotic cells where it is used in sugar and amino acid uptake. ATP, rather than proton motive force, usually drives sodium transport in eucaryotic cells.

Often a microorganism has more than one transport system for each nutrient, as can be seen with *E. coli.* This bacterium has at least five transport systems for the sugar galactose, three systems each for the amino acids glutamate and leucine, and two potassium transport complexes. When there are several transport systems for the same substance, the systems differ in such properties as their energy source, their affinity for the solute transported, and the nature of their regulation. Presumably this diversity gives its possessor an added competitive advantage in a variable environment.

Group Translocation

In active transport, solute molecules move across a membrane without modification. Many procaryotes also take up molecules by **group translocation,** a process in which a molecule is transported into the cell while being chemically altered (this can be classified as a type of energy-dependent transport because metabolic energy is used). The best-known group translocation system is the **phosphoenolpyruvate: sugar phosphotransferase system (PTS).** It transports a variety of sugars into procaryotic cells while phosphorylating them using phosphoenolpyruvate (PEP) as the phosphate donor.

$$\text{PEP} + \text{sugar (outside)} \rightarrow \text{pyruvate} + \text{sugar} - \text{P (inside)}$$

The PTS is quite complex. In *E. coli* and *Salmonella typhimurium,* it consists of two enzymes and a low molecular weight heat-stable protein (HPr). HPr and enzyme I (EI) are cytoplasmic. Enzyme II (EII) is more variable in structure and often composed of three subunits or domains. EIIA (formerly called EIII) is cytoplasmic and soluble. EIIB also is hydrophilic but frequently is attached to EIIC, a hydrophobic protein that is embedded in the membrane. A high-energy phosphate is transferred from PEP to enzyme II with the aid of enzyme I and HPr (**figure 5.5**). Then, a sugar molecule is phosphorylated as it is carried across the membrane by enzyme II. Enzyme II transports only specific sugars and varies with PTS, whereas enzyme I and HPr are common to all PTSs.

PTSs are widely distributed in procaryotes. Except for some species of *Bacillus* that have both glycolysis and the phosphotransferase system, aerobic bacteria seem to lack PTSs. Members of the

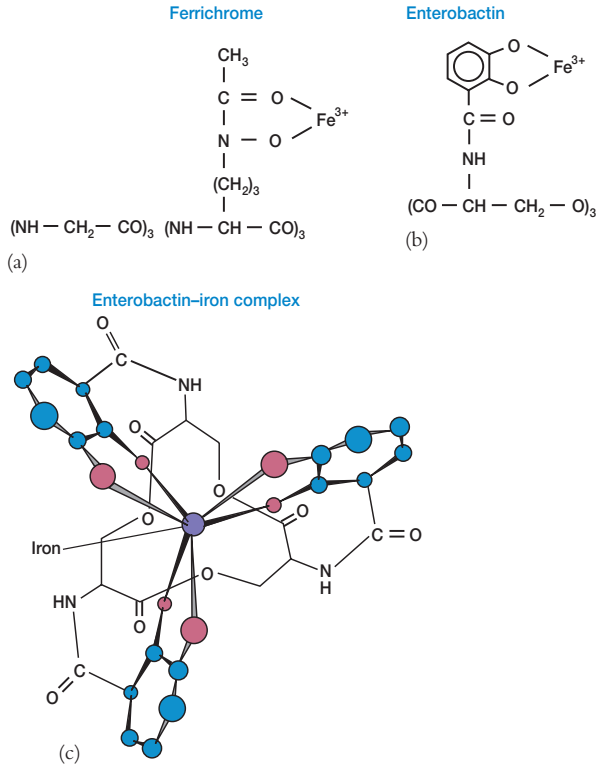


Figure 5.6 Siderophore Ferric Iron Complexes. (**a**) Ferrichrome is a cyclic hydroxamate [—CO—N(O$^-$)—] molecule formed by many fungi. (**b**) *E. coli* produces the cyclic catecholate derivative, enterobactin. (**c**) Ferric iron probably complexes with three siderophore groups to form a six-coordinate, octahedral complex as shown in this illustration of the enterobactin-iron complex.

genera *Escherichia, Salmonella, Staphylococcus,* and other facultatively anaerobic bacteria (*see p. 127*) have phosphotransferase systems; some obligately anaerobic bacteria (e.g., *Clostridium*) also have PTSs. Many carbohydrates are transported by these systems. *E. coli* takes up glucose, fructose, mannitol, sucrose, *N*-acetylglucosamine, cellobiose, and other carbohydrates by group translocation. Besides their role in transport, PTS proteins can act as chemoreceptors for chemotaxis.

Iron Uptake

Almost all microorganisms require iron for use in cytochromes and many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric iron (Fe^{3+}) and its derivatives, which leaves little free iron available for transport. Many bacteria and fungi have overcome this difficulty by secreting siderophores [Greek for iron bearers]. **Siderophores** are low molecular weight molecules that are able to complex with ferric iron and supply it to the cell. These iron-transport molecules are normally either hydroxamates or phenolates-catecholates. Ferrichrome is a hydroxamate produced by many fungi; enterobactin is the catecholate formed by *E. coli* (**figure 5.6*a,b***). It appears that three siderophore groups complex with iron orbitals to form a six-coordinate, octahedral complex (figure 5.6*c*).

Microorganisms secrete siderophores when little iron is available in the medium. Once the iron-siderophore complex has reached the cell surface, it binds to a siderophore-receptor protein. Then the iron is either released to enter the cell directly or the whole iron-siderophore complex is transported inside by an ABC transporter. In *E. coli* the siderophore receptor is in the outer membrane of the cell envelope; when the iron reaches the periplasmic space, it moves through the plasma membrane with the aid of the transporter. After the iron has entered the cell, it is reduced to the ferrous form (Fe^{2+}). Iron is so crucial to microorganisms that they may use more than one route of iron uptake to ensure an adequate supply.

1. Describe facilitated diffusion, active transport, and group translocation in terms of their distinctive characteristics and mechanisms.
2. How do binding protein transport systems and membrane-bound transport systems differ with respect to energy sources? What are symport and antiport processes?
3. How are siderophores involved in iron transport?

5.7 Culture Media

Much of the study of microbiology depends on the ability to grow and maintain microorganisms in the laboratory, and this is possible only if suitable culture media are available. A culture medium is a solid or liquid preparation used to grow, transport, and store microorganisms. To be effective, the medium must contain all the nutrients the microorganism requires for growth. Specialized media are essential in the isolation and identification of microorganisms, the testing of antibiotic sensitivities, water and food analysis, industrial microbiology, and other activities. Although all microorganisms need sources of energy, carbon, nitrogen, phosphorus, sulfur, and various minerals, the precise composition of a satisfactory medium will depend on the species one is trying to cultivate because nutritional requirements vary so greatly. Knowledge of a microorganism's normal habitat often is useful in selecting an appropriate culture medium because its nutrient requirements reflect its natural surroundings. Frequently a medium is used to select and grow specific microorganisms or to help identify a particular species. In such cases the function of the medium also will determine its composition.

Synthetic or Defined Media

Some microorganisms, particularly photolithotrophic autotrophs such as cyanobacteria and eucaryotic algae, can be grown on relatively simple media containing CO_2 as a carbon source (often added as sodium carbonate or bicarbonate), nitrate or ammonia as a nitrogen source, sulfate, phosphate, and a variety of minerals

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 (Na_2Mg 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 $Co(NO_3)_2 \cdot 6H_2O$.

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

(**table 5.4**). Such a medium in which all components are known is a **defined medium** or **synthetic medium.** Many chemoorganotrophic heterotrophs also can be grown in defined media with glucose as a carbon source and an ammonium salt as a nitrogen source. Not all defined media are as simple as the examples in table 5.4 but may be constructed from dozens of components. Defined media are used widely in research, as it is often desirable to know what the experimental microorganism is metabolizing.

Complex Media

Media that contain some ingredients of unknown chemical composition are **complex media.** Such media are very useful, as a single complex medium may be sufficiently rich and complete to meet the nutritional requirements of many different microorganisms. In addition, complex media often are needed because the nutritional requirements of a particular microorganism are unknown, and thus a defined medium cannot be constructed. This is the situation with many fastidious bacteria, some of which may even require a medium containing blood or serum.

Complex media contain undefined components like peptones, meat extract, and yeast extract. **Peptones** are protein hydrolysates prepared by partial proteolytic digestion of meat, casein, soya meal, gelatin, and other protein sources. They serve as sources of carbon, energy, and nitrogen. Beef extract and yeast extract are aqueous extracts of lean beef and brewer's yeast, respectively. Beef extract contains amino acids, peptides, nucleotides, organic acids, vitamins, and minerals. Yeast extract is an excellent source of B vitamins as well as nitrogen and carbon compounds. Three commonly used complex media are (1) nutrient broth, (2) tryptic soy broth, and (3) MacConkey agar (**table 5.5**).

If a solid medium is needed for surface cultivation of microorganisms, liquid media can be solidified with the addition of 1.0 to 2.0% agar; most commonly 1.5% is used. **Agar** is a sulfated polymer composed mainly of D-galactose, 3,6-anhydro-L-galactose, and D-glucuronic acid (**Box 5.1**). It usually is extracted from red algae (*see figure 26.8*). Agar is well suited as a solidifying agent because after it has been melted in boiling water, it can be cooled to about 40 to 42°C before hardening and will not melt again until the temperature rises to about 80 to 90°C. Agar is also an excellent hardening agent because most microorganisms cannot degrade it.

Other solidifying agents are sometimes employed. For example, silica gel is used to grow autotrophic bacteria on solid media in the absence of organic substances and to determine carbon sources for heterotrophic bacteria by supplementing the medium with various organic compounds.

Types of Media

Media such as tryptic soy broth and tryptic soy agar are called general purpose media because they support the growth of many microorganisms. Blood and other special nutrients may be added to general purpose media to encourage the growth of fastidious heterotrophs. These specially fortified media (e.g., blood agar) are called enriched media.

Selective media favor the growth of particular microorganisms. Bile salts or dyes like basic fuchsin and crystal violet favor the growth of gram-negative bacteria by inhibiting the growth of gram-positive bacteria without affecting gram-negative organisms. Endo agar, eosin methylene blue agar, and MacConkey agar

Box 5.1

The Discovery of Agar as a Solidifying Agent and the Isolation of Pure Cultures

The earliest culture media were liquid, which made the isolation of bacteria to prepare pure cultures extremely difficult. In practice, a mixture of bacteria was diluted successively until only one organism, as an average, was present in a culture vessel. If everything went well, the individual bacterium thus isolated would reproduce to give a pure culture. This approach was tedious, gave variable results, and was plagued by contamination problems. Progress in isolating pathogenic bacteria understandably was slow.

The development of techniques for growing microorganisms on solid media and efficiently obtaining pure cultures was due to the efforts of the German bacteriologist Robert Koch and his associates. In 1881 Koch published an article describing the use of boiled potatoes, sliced with a flame-sterilized knife, in culturing bacteria. The surface of a sterile slice of potato was inoculated with bacteria from a needle tip, and then the bacteria were streaked out over the surface so that a few individual cells would be separated from the remainder. The slices were incubated beneath bell jars to prevent airborne contamination, and the isolated cells developed into pure colonies. Unfortunately many bacteria would not grow well on potato slices.

At about the same time, Frederick Loeffler, an associate of Koch's, developed a meat extract peptone medium for cultivating pathogenic bacteria. Koch decided to try solidifying this medium. Koch was an amateur photographer—he was the first to take photomicrographs of bacteria—and was experienced in preparing his own photographic plates from silver salts and gelatin. Precisely the same approach was employed for preparing solid media. He spread a mixture of Loeffler's medium and gelatin over a glass plate, allowed it to harden, and inoculated the surface in the same way he had inoculated his sliced potatoes. The new solid medium worked well, but it could not be incubated at 37°C (the best temperature for most human bacterial pathogens) because the gelatin would melt. Furthermore, some bacteria digested the gelatin.

About a year later, in 1882, agar was first used as a solidifying agent. It had been discovered by a Japanese innkeeper, Minora Tarazaemon. The story goes that he threw out extra seaweed soup and discovered the next day that it had jelled during the cold winter night. Agar had been used by the East Indies Dutch to make jellies and jams. Fannie Eilshemius Hesse (*see figure 1.5*), the New Jersey–born wife of Walther Hesse, one of Koch's assistants, had learned of agar from a Dutch acquaintance and suggested its use when she heard of the difficulties with gelatin. Agar-solidified medium was an instant success and continues to be essential in all areas of microbiology.

(**table 5.5**), three media widely used for the detection of *E. coli* and related bacteria in water supplies and elsewhere, contain dyes that suppress gram-positive bacterial growth. MacConkey agar also contains bile salts. Bacteria also may be selected by incubation with nutrients that they specifically can use. A medium containing only cellulose as a carbon and energy source is quite effective in the isolation of cellulose-digesting bacteria. The possibilities for selection are endless, and there are dozens of special selective media in use.

Differential media are media that distinguish between different groups of bacteria and even permit tentative identification of microorganisms based on their biological characteristics. Blood agar is both a differential medium and an enriched one. It distinguishes between hemolytic and nonhemolytic bacteria. Hemolytic bacteria (e.g., many streptococci and staphylococci isolated from throats) produce clear zones around their colonies because of red blood cell destruction. MacConkey agar is both differential and selective. Since it contains lactose and neutral red dye, lactose-fermenting colonies appear pink to red in color and are easily distinguished from colonies of nonfermenters.

1. Describe the following kinds of media and their uses: defined or synthetic media, complex media, general purpose media, enriched media, selective media, and differential media. Give an example of each kind.
2. What are peptones, yeast extract, beef extract, and agar? Why are they used in media?

5.8 Isolation of Pure Cultures

In natural habitats microorganisms usually grow in complex, mixed populations containing several species. This presents a problem for the microbiologist because a single type of microorganism cannot be studied adequately in a mixed culture. One needs a **pure culture,** a population of cells arising from a single cell, to characterize an individual species. Pure cultures are so important that the development of pure culture techniques by the German bacteriologist Robert Koch transformed microbiology. Within about 20 years after the development of pure culture techniques most pathogens responsible for the major human bacterial diseases had been isolated (*see Table 1.1*). There are several ways to prepare pure cultures; a few of the more common approaches are reviewed here. A brief survey of some major milestones in microbiology (chapter 1)

The Spread Plate and Streak Plate

If a mixture of cells is spread out on an agar surface so that every cell grows into a completely separate **colony,** a macroscopically visible growth or cluster of microorganisms on a solid medium, each colony represents a pure culture. The **spread plate** is an easy, direct way of achieving this result. A small volume of dilute microbial mixture containing around 30 to 300 cells is transferred to the center of an agar plate and spread evenly over the surface with a sterile bent-glass rod (**figure 5.7**). The dispersed cells develop into isolated colonies. Because the number of colonies

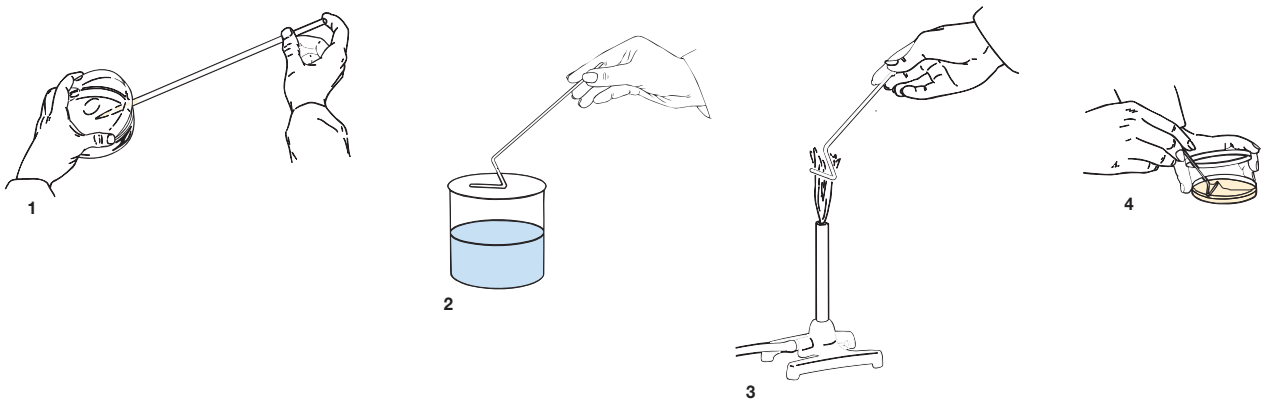


Figure 5.7 Spread-Plate Technique. The preparation of a spread plate. (*1*) Pipette a small sample onto the center of an agar medium plate. (*2*) Dip a glass spreader into a beaker of ethanol. (3) Briefly flame the ethanol-soaked spreader and allow it to cool. (*4*) Spread the sample evenly over the agar surface with the sterilized spreader. Incubate.

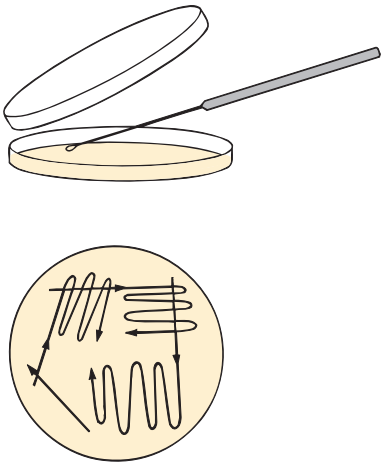


Figure 5.8 Streak-Plate Technique. Preparation of streak plates. The upper illustration shows a petri dish of agar being streaked with an inoculating loop. A commonly used streaking pattern is pictured at the bottom.

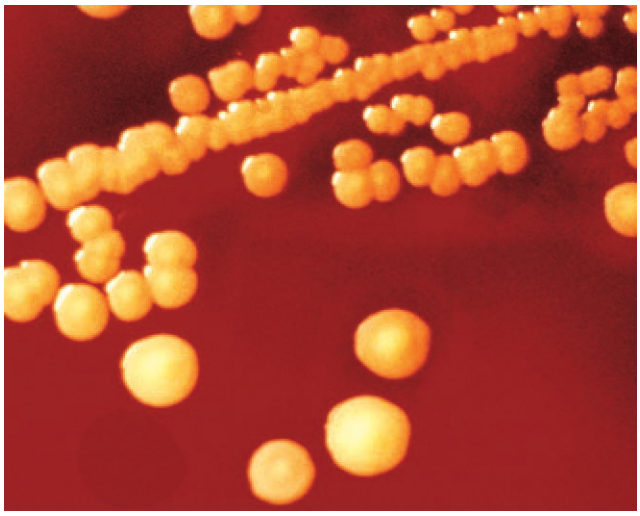


Figure 5.9 Bacterial Colonies on Agar. Colonies growing on a streak plate. A blood-agar plate has been inoculated with *Staphylococcus aureus.* After incubation, large, golden colonies have formed on the agar.

should equal the number of viable organisms in the sample, spread plates can be used to count the microbial population.

Pure colonies also can be obtained from **streak plates.** The microbial mixture is transferred to the edge of an agar plate with an inoculating loop or swab and then streaked out over the surface in one of several patterns (**figure 5.8**). At some point in the process, single cells drop from the loop as it is rubbed along the agar surface and develop into separate colonies (**figure 5.9**). In both spread-plate and streak-plate techniques, successful isolation depends on spatial separation of single cells.

The Pour Plate

Extensively used with bacteria and fungi, a **pour plate** also can yield isolated colonies. The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies when plating (**figure 5.10**). Then small volumes of several diluted samples are mixed with liquid agar that has been cooled to about 45°C, and the mixtures are poured immediately into sterile culture dishes. Most bacteria and fungi are not killed by a brief exposure to the warm agar. After the agar has hardened, each cell is fixed in place and forms an individual

Box 5.2

The Enrichment and Isolation of Pure Cultures

A major practical problem is the preparation of pure cultures when microorganisms are present in very low numbers in a sample. Plating methods can be combined with the use of selective or differential media to enrich and isolate rare microorganisms. A good example is the isolation of bacteria that degrade the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Bacteria able to metabolize 2,4-D can be obtained with a liquid medium containing 2,4-D as its sole carbon source and the required nitrogen, phosphorus, sulfur, and mineral components. When this medium is inoculated with soil, only bacteria able to use 2,4-D will grow. After incubation, a sample of the original culture is transferred to a fresh flask of selective medium for further enrichment of 2,4-D metabolizing bacteria. A mixed population of 2,4-D degrading bacteria will arise after several such transfers. Pure cultures can be obtained by plating this mixture on agar containing 2,4-D as the sole carbon source. Only bacteria able to grow on 2,4-D form visible colonies and can be subcultured. This same general approach is used to isolate and purify a variety of bacteria by selecting for specific physiological characteristics.

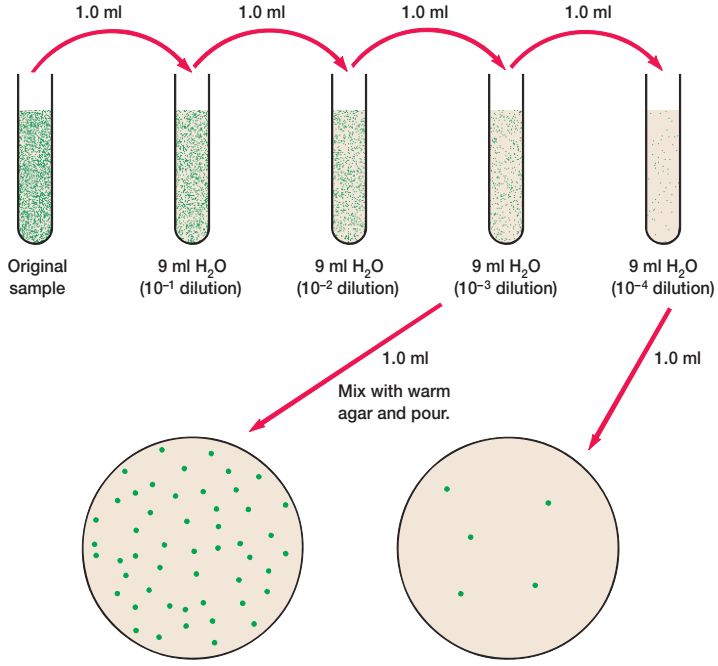


Figure 5.10 **The Pour-Plate Technique.** The original sample is diluted several times to thin out the population sufficiently. The most diluted samples are then mixed with warm agar and poured into petri dishes. Isolated cells grow into colonies and can be used to establish pure cultures. The surface colonies are circular; subsurface colonies would be lenticular or lens shaped.

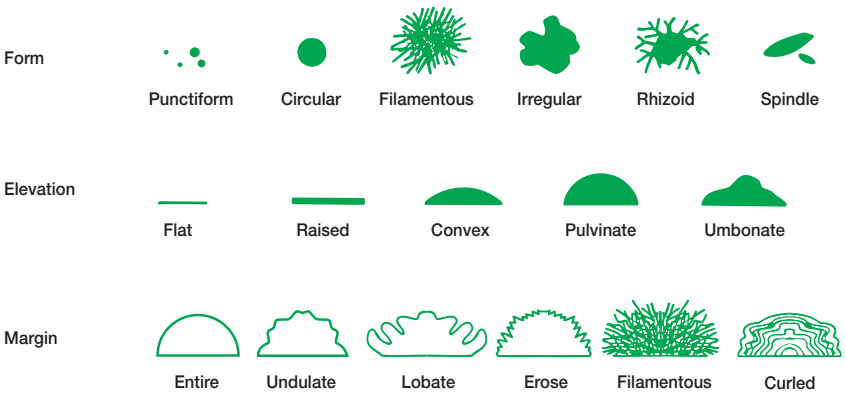
colony. Plates containing between 30 and 300 colonies are counted. The total number of colonies equals the number of viable microorganisms in the diluted sample. Colonies growing on the surface also can be used to inoculate fresh medium and prepare pure cultures (**Box 5.2**).

The preceding techniques require the use of special culture dishes named **petri dishes** or plates after their inventor Julius Richard Petri, a member of Robert Koch's laboratory; Petri developed these dishes around 1887 and they immediately replaced agar-coated glass plates. They consist of two round halves, the top half overlapping the bottom (figure 5.8). Petri dishes are very easy to use, may be stacked on each other to save space, and are one of the most common items in microbiology laboratories.

Colony Morphology and Growth

Colony development on agar surfaces aids the microbiologist in identifying bacteria because individual species often form colonies of characteristic size and appearance (**figure 5.11**). When a mixed population has been plated properly, it sometimes is possible to identify the desired colony based on its overall appearance and use it to obtain a pure culture. The structure of bacterial colonies also has been examined with the scanning electron microscope. The microscopic structure of colonies is often as variable as their visible appearance (**figure 5.12**).

In nature bacteria and many other microorganisms often grow on surfaces in biofilms. However, sometimes they do form discrete colonies. Therefore an understanding of colony growth is impor-



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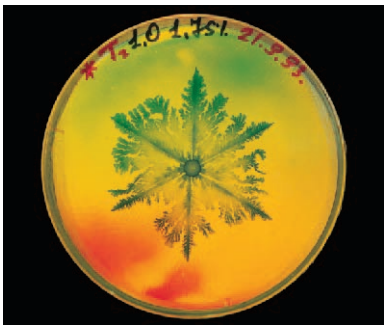
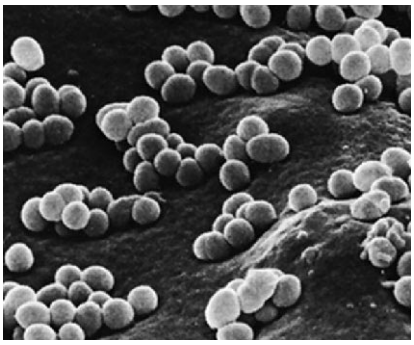


(b)

Figure 5.11 Bacterial Colony Morphology. (**a**) Variations in bacterial colony morphology seen with the naked eye. The general form of the colony and the shape of the edge or margin can be determined by looking down at the top of the colony. The nature of colony elevation is apparent when viewed from the side as the plate is held at eye level. (**b**) Colony morphology can vary dramatically with the medium on which the bacteria are growing. These beautiful snowflakelike colonies were formed by *Bacillus subtilis* growing on nutrient-poor agar. The bacteria apparently behave cooperatively when confronted with poor growth conditions, and often the result is an intricate structure that resembles the fractal patterns seen in nonliving systems.



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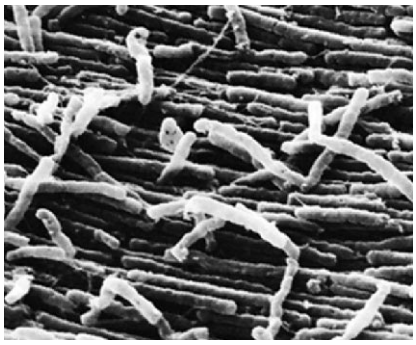

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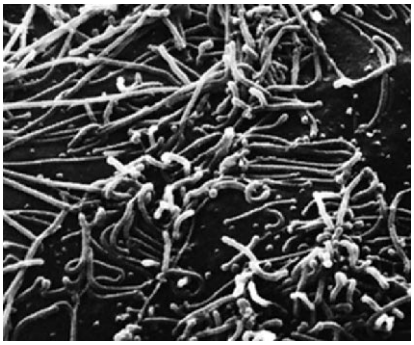

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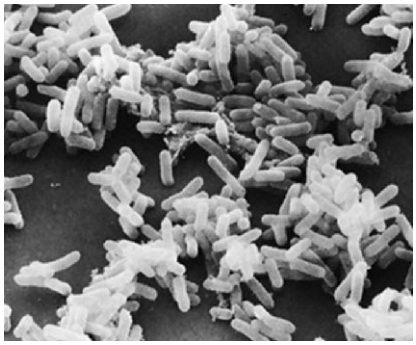

(d)

Figure 5.12 Scanning Electron Micrographs of Bacterial Colonies. (**a**) *Micrococcus* on agar (×31,000). (**b**) *Clostridium* (×12,000). (**c**) *Mycoplasma pneumoniae* (×26,000). (**d**) *Escherichia coli* (×14,000).

tant, and the growth of colonies on agar has been frequently studied. Generally the most rapid cell growth occurs at the colony edge. Growth is much slower in the center, and cell autolysis takes place in the older central portions of some colonies. These differences in growth appear due to gradients of oxygen, nutrients, and toxic products within the colony. At the colony edge, oxygen and nutrients are plentiful. The colony center, of course, is much thicker than the edge. Consequently oxygen and nutrients do not diffuse readily into the center, toxic metabolic products cannot be quickly eliminated, and growth in the colony center is slowed or stopped. Because of these environmental variations within a colony, cells on the periphery can be growing at maximum rates while cells in the center are dying. Biofilms (pp. 620–22)

It is obvious from the colonies pictured in figure 5.11 that bacteria growing on solid surfaces such as agar can form quite complex and intricate colony shapes. These patterns vary with nutrient availability and the hardness of the agar surface. It is not yet clear how characteristic colony patterns develop. Nutrient diffusion and availability, bacterial chemotaxis, and the presence of liquid on the surface all appear to play a role in pattern formation. Undoubtedly cell-cell communication and quorum sensing (*see pp. 132–33*) is important as well. Much work will be required to understand the formation of bacterial colonies and biofilms.

1. What are pure cultures, and why are they important? How are spread plates, streak plates, and pour plates prepared?
2. In what way does microbial growth vary within a colony? What factors might cause these variations in growth?

Summary

1. Microorganisms require nutrients, materials that are used in biosynthesis and energy production.
2. Macronutrients or macroelements (C, O, H, N, S, P, K, Ca, Mg, and Fe) are needed in relatively large quantities; micronutrients or trace elements (e.g., Mn, Zn, Co, Mo, Ni, and Cu) are used in very small amounts.
3. Autotrophs use CO_2 as their primary or sole carbon source; heterotrophs employ organic molecules.
4. Microorganisms can be classified based on their energy and electron sources (**table 5.1**). Phototrophs use light energy, and chemotrophs obtain energy from the oxidation of chemical compounds. Electrons are extracted from reduced inorganic substances by lithotrophs and from organic compounds by organotrophs (**table 5.2**).
5. Nitrogen, phosphorus, and sulfur may be obtained from the same organic molecules that supply carbon, from the direct incorporation of ammonia and phosphate, and by the reduction and assimilation of oxidized inorganic molecules.
6. Probably most microorganisms need growth factors. Growth factor requirements make microbiological assays possible.
7. Although some nutrients can enter cells by passive diffusion, a membrane carrier protein is usually required.
8. In facilitated diffusion the transport protein simply carries a molecule across the membrane in the direction of decreasing concentration, and no metabolic energy is required (**figure 5.2**).
9. Active transport systems use metabolic energy and membrane carrier proteins to concentrate substances actively by transporting them against a gradient. ATP is used as an energy source by ABC transporters (**figure 5.3**). Gradients of protons and sodium ions also drive solute uptake across membranes (**figure 5.4**).
10. Bacteria also transport organic molecules while modifying them, a process known as group translocation. For example, many sugars are transported and phosphorylated simultaneously (**figure 5.5**).
11. Iron is accumulated by the secretion of siderophores, small molecules able to complex with ferric iron (**figure 5.6**). When the iron-siderophore complex reaches the cell surface, it is taken inside and the iron is reduced to the ferrous form.
12. Culture media can be constructed completely from chemically defined components (defined media or synthetic media) or may contain constituents like peptones and yeast extract whose precise composition is unknown (complex media).
13. Culture media can be solidified by the addition of agar, a complex polysaccharide from red algae.
14. Culture media are classified based on function and composition as general purpose media, enriched media, selective media, and differential media.
15. Pure cultures usually are obtained by isolating individual cells with any of three plating techniques: the spread-plate, streak-plate, and pour-plate methods (**figures 5.7** and **5.8**).
16. Microorganisms growing on solid surfaces tend to form colonies with distinctive morphology. Colonies usually grow most rapidly at the edge where larger amounts of required resources are available.

Key Terms

active transport *101*
agar *105*
antiport *102*
ATP-binding cassette transporters (ABC transporters) *101*
autotrophs *96*
chemoheterotrophs *98*
chemolithotrophic autotrophs *98*
chemoorganotrophic heterotrophs *98*
chemotrophs *97*
colony *106*
complex medium *105*
defined medium *105*
differential media *106*
facilitated diffusion *100*
group translocation *103*
growth factors *99*
heterotrophs *96*
lithotrophs *97*
macroelements *96*
micronutrients *96*
mixotrophic *98*
nutrient *96*
organotrophs *97*
passive diffusion *100*
peptones *105*
permease *100*

petri dish *108*
phosphoenolpyruvate: sugar phosphotransferase system (PTS) *103*
photoautotrophs *97*
photolithotrophic autotrophs *97*
photoorganotrophic heterotrophs *98*
phototrophs *97*
pour plate *107*
pure culture *106*
selective media *105*
siderophores *104*
spread plate *106*
streak plate *107*
symport *102*
synthetic medium *105*
trace elements *96*
vitamins *99*

Questions for Thought and Review

1. Why is it so difficult to demonstrate the micronutrient requirements of microorganisms?
2. List some of the most important uses of the nitrogen, phosphorus, and sulfur that microorganisms obtain from their surroundings.
3. Why are amino acids, purines, and pyrimidines often growth factors, whereas glucose is usually not?
4. Why do microorganisms normally take up nutrients using transport proteins or permeases? What advantage does a microorganism gain by employing active transport rather than facilitated diffusion?
5. If you wished to obtain a pure culture of bacteria that could degrade benzene and use it as a carbon and energy source, how would you proceed?
6. Describe the nutritional requirements of a chemolithotrophic heterotroph. Where might you search for such a bacterium?
7. Suppose that you carry out a serial dilution of a 0.1 ml sample as shown in figure 5.10. The 10^{-3} plate gives 80 colonies and the 10^{-4} plate yields four colonies. Calculate the concentration (bacteria/ml) of the original, undiluted sample.

Critical Thinking Questions

1. Discuss the advantages and disadvantages of group translocation versus endocytosis for the host cell.
2. Explain why isolation of a pure culture on selective solid medium may not be successful.

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