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Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996.

Chapter 4 Bacterial Metabolism

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General Concepts

Heterotrophic Metabolism

Heterotrophic metabolism is the biologic oxidation of organic compounds, such as glucose, to yield ATP and simpler organic (or inorganic) compounds, which are needed by the bacterial cell for biosynthetic or assimilatory reactions.

Respiration

Respiration is a type of heterotrophic metabolism that uses oxygen and in which 38 moles of ATP are derived from the oxidation of 1 mole of glucose, yielding 380,000 cal. (An additional 308,000 cal is lost as heat.)

Fermentation

In fermentation, another type of heterotrophic metabolism, an organic compound rather than oxygen is the terminal electron (or hydrogen) acceptor. Less energy is generated from this incomplete form of glucose oxidation, but the process supports anaerobic growth.

Krebs Cycle

The Krebs cycle is the oxidative process in respiration by which pyruvate (via acetyl coenzyme A) is completely decarboxylated to CO_2. The pathway yields 15 moles of ATP (150,000 calories).

Glyoxylate Cycle

The glyoxylate cycle, which occurs in some bacteria, is a modification of the Krebs cycle. Acetyl coenzyme A is generated directly from oxidation of fatty acids or other lipid compounds.

Electron Transport and Oxidative Phosphorylation

In the final stage of respiration, ATP is formed through a series of electron transfer reactions within the cytoplasmic membrane that drive the oxidative phosphorylation of ADP to ATP. Bacteria use various flavins, cytochrome, and non-heme iron components as well as multiple cytochrome oxidases for this process.

Mitchell or Proton Extrusion Hypothesis

The Mitchell hypothesis explains the energy conservation in all cells on the basis of the selective extrusion of H^+ ions across a proton-impermeable membrane, which generates a proton motive force. This energy allows for ATP synthesis both in respiration and photosynthesis.

Bacterial Photosynthesis

Bacterial photosynthesis is a light-dependent, anaerobic mode of metabolism. Carbon dioxide is reduced to glucose, which is used for both biosynthesis and energy production. Depending on the hydrogen source used to reduce CO_2, both photolithotrophic and photoorganotrophic reactions exist in bacteria.

Autotrophy

Autotrophy is a unique form of metabolism found only in bacteria. Inorganic compounds are oxidized directly (without using sunlight) to yield energy (e.g., NH_3, NO_2^-, S_2, and Fe^{2+}). This metabolic mode also requires energy for CO_2 reduction, like photosynthesis, but no lipid-mediated processes are involved. This metabolic mode has also been called chemotrophy, chemoautotrophy, or chemolithotrophy.

Anaerobic Respiration

Anaerobic respiration is another heterotrophic mode of metabolism in which a specific compound other than O_2 serves as a terminal electron acceptor. Such acceptor compounds include NO_3^-, SO_4^{2-}, fumarate, and even CO_2 for methane-producing bacteria.

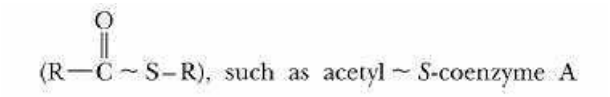
The Nitrogen Cycle

The nitrogen cycle consists of a recycling process by which organic and inorganic nitrogen compounds are used metabolically and recycled among bacteria, plants, and animals. Important processes, including ammonification, mineralization, nitrification, denitrification, and nitrogen fixation, are carried out primarily by bacteria.

Introduction

Metabolism refers to all the biochemical reactions that occur in a cell or organism. The study of bacterial metabolism focuses on the chemical diversity of substrate oxidations and dissimilation reactions (reactions by which substrate molecules are broken down), which normally function in bacteria to generate energy. Also within the scope of bacterial metabolism is the study of the uptake and utilization of the inorganic or organic compounds required for growth and maintenance of a cellular steady state (assimilation reactions). These respective exergonic (energy-yielding) and endergonic (energy-requiring) reactions are catalyzed within the living bacterial cell by integrated enzyme systems, the end result being self-replication of the cell. The capability of microbial cells to live, function, and replicate in an appropriate chemical milieu (such as a bacterial culture medium) and the chemical changes that result during this transformation constitute the scope of bacterial metabolism.

The bacterial cell is a highly specialized energy transformer. Chemical energy generated by substrate oxidations is conserved by formation of high-energy compounds such as adenosine diphosphate (ADP) and adenosine triphosphate (ATP) or compounds containing the thioester bond



(acetyl ~ SCoA) or succinyl ~ SCoA. ADP and ATP represent adenosine monophosphate (AMP) plus one and two high-energy phosphates (AMP ~ P and AMP ~ P~ P, respectively); the energy is stored in these compounds as high-energy phosphate bonds. In the presence of proper enzyme systems, these compounds can be used as energy sources to synthesize the new complex organic compounds needed by the cell. All living cells must maintain steady-state biochemical reactions for the formation and use of such high-energy compounds.

Kluyver and Donker (1924 to 1926) recognized that bacterial cells, regardless of species, were in many respects similar chemically to all other living cells. For example, these investigators recognized that hydrogen transfer is a common and fundamental feature of all metabolic processes. Bacteria, like mammalian and plant cells, use ATP or the high-energy phosphate bond (~ P) as the primary chemical energy source. Bacteria also require the B-complex vitamins as functional coenzymes for many oxidation-reduction reactions needed for growth and energy transformation. An organism such as *Thiobacillus thiooxidans*, grown in a medium containing only sulfur and inorganic salts, synthesizes large amounts of thiamine, riboflavine, nicotinic acid, pantothenic acid, pyridoxine, and biotin. Therefore, Kluyver proposed the unity theory of biochemistry (*Die Einheit in der Biochemie*), which states that all basic enzymatic reactions which support and maintain life processes within cells of organisms, had more similarities than differences. This concept of biochemical unity stimulated many investigators to use bacteria as model systems for studying related eukaryotic, plant and animal biochemical reactions that are essentially "identical" at the molecular level.

From a nutritional, or metabolic, viewpoint, three major physiologic types of bacteria exist: the heterotrophs (or chemoorganotrophs), the autotrophs (or chemolithotrophs), and the photosynthetic bacteria (or phototrophs) (Table 4-1). These are discussed below.

Table 4-1

Nutritional Diversity Exhibited by Physiologically Different Bacteria.

Heterotrophic Metabolism

Heterotrophic bacteria, which include all pathogens, obtain energy from oxidation of organic compounds. Carbohydrates (particularly glucose), lipids, and protein are the most commonly oxidized compounds. Biologic oxidation of these organic compounds by bacteria results in synthesis of ATP as the chemical energy source. This process also permits generation of simpler organic compounds (precursor molecules) needed by the bacteria cell for biosynthetic or assimilatory reactions.

The Krebs cycle intermediate compounds serve as precursor molecules (building blocks) for the energy-requiring biosynthesis of complex organic compounds in bacteria. Degradation reactions that simultaneously produce energy and generate precursor molecules for the biosynthesis of new cellular constituents are called amphibolic.

All heterotrophic bacteria require preformed organic compounds. These carbon- and nitrogen-containing compounds are growth substrates, which are used aerobically or anaerobically to generate reducing equivalents (e.g., reduced nicotinamide adenine dinucleotide; NADH + H^+); these reducing equivalents in turn are chemical energy sources for all biologic oxidative and fermentative systems. Heterotrophs are the most commonly studied bacteria; they grow readily in media containing carbohydrates, proteins, or other complex nutrients such as blood. Also, growth media may be enriched by the addition of other naturally occurring compounds such as milk (to study lactic acid bacteria) or hydrocarbons (to study hydrocarbon-oxidizing organisms).

Respiration

Glucose is the most common substrate used for studying heterotrophic metabolism. Most aerobic organisms oxidize glucose completely by the following reaction equation:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{energy}$$

This equation expresses the cellular oxidation process called respiration. Respiration occurs within the cells of plants and animals, normally generating 38 ATP molecules (as energy) from the oxidation of 1 molecule of glucose. This yields approximately 380,000 calories (cal) per mode of glucose (ATP ~ 10,000 cal/mole). Thermodynamically, the complete oxidation of one mole of glucose should yield approximately 688,000 cal; the energy that is not conserved biologically as chemical energy (or ATP formation) is liberated as heat (308,000 cal). Thus, the cellular respiratory process is at best about 55% efficient.

Glucose oxidation is the most commonly studied dissimilatory reaction leading to energy production or ATP synthesis. The complete oxidation of glucose may involve three fundamental biochemical pathways. The first is the glycolytic or Embden- Meyerhof-Parnas pathway (Fig. 4-1), the second is the Krebs cycle (also called the citric acid cycle or tricarboxylic acid cycle), and the third is the series of membrane-bound electron transport oxidations coupled to oxidative phosphorylation.

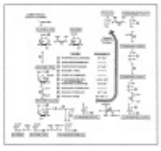


Figure 4-1

Glycolytic (EMP) pathway.

Respiration takes place when any organic compound (usually carbohydrate) is oxidized completely to CO_2 and H_2O. In aerobic respiration, molecular O_2serves as the terminal acceptor of electrons. For anaerobic respiration, NO_3^-, SO_4^{2-}, CO_2, or fumarate can serve as terminal electron acceptors (rather than O_2), depending on the bacterium studied. The end result of the respiratory process is the complete oxidation of the organic substrate molecule, and the end products formed are primarily CO_2 and H_2O. Ammonia is formed also if protein (or amino acid) is the substrate oxidized. The biochemical pathways normally

involved in oxidation of various naturally occurring organic compounds are summarized in Figure 4-2.

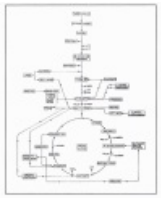


Figure 4-2

Heterotrophic metabolism, general pathway.

Metabolically, bacteria are unlike cyanobacteria (blue-green algae) and eukaryotes in that glucose oxidation may occur by more than one pathway. In bacteria, glycolysis represents one of several pathways by which bacteria can catabolically attack glucose. The glycolytic pathway is most commonly associated with anaerobic or fermentative metabolism in bacteria and yeasts. In bacteria, other minor heterofermentative pathways, such as the phosphoketolase pathway, also exist.

In addition, two other glucose-catabolizing pathways are found in bacteria: the oxidative pentose phosphate pathway (hexose monophosphate shunt), (Fig. 4-3) and the Entner-Doudoroff pathway, which is almost exclusively found in obligate aerobic bacteria (Fig. 4-4). The highly oxidative *Azotobacter* and most *Pseudomonas* species, for example, utilize the Entner-Doudoroff pathway for glucose catabolism, because these organisms lack the enzyme phosphofructokinase and hence cannot synthesize fructose 1,6-diphosphate, a key intermediate compound in the glycolytic pathway. (Phospho-fructokinase is also sensitive to molecular O_2 and does not function in obligate aerobes). Other bacteria, which lack aldolase (which splits fructose-1,6-diphosphate into two triose phosphate compounds), also cannot have a functional glycolytic pathway. Although the Entner-Doudoroff pathway is usually associated with obligate aerobic bacteria, it is present in the facultative anaerobe *Zymomonas mobilis* (formerly *Pseudomonas lindneri*). This organism dissimilates glucose to ethanol and represents a major alcoholic fermentation reaction in a bacterium.

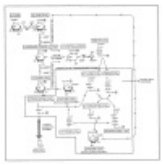


Figure 4-3

Hexose monophosphate (HMS) pathway.

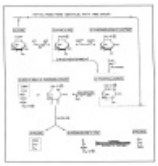


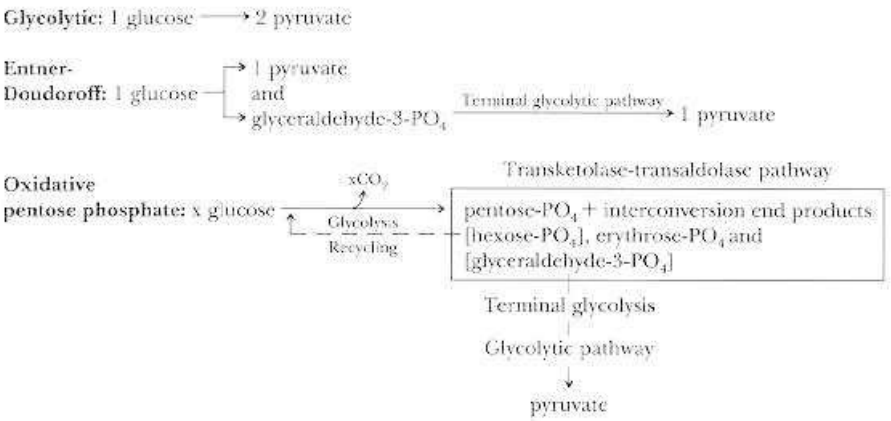
Figure 4-4

Entner-Doudoroff (ED) pathway.

Glucose dissimilation also occurs by the hexose monophosphate shunt (Fig. 4-3). This oxidative pathway was discovered in tissues that actively metabolize glucose in the presence of two glycolytic pathway inhibitors (iodoacetate and fluoride). Neither inhibitor had an effect on glucose dissimilation, and NADPH + H^+ generation occurred directly from the oxidation of glucose-6-phosphate (to 6-phosphoglucono-δ-lactone) by glucose-6phosphate dehydrogenase. The pentose phosphate pathway subsequently permits the direct oxidative decarboxylation of glucose to pentoses. The capability of this oxidative metabolic system to bypass glycolysis explains the term shunt.

The biochemical reactions of the Entner-Doudoroff pathway are a modification of the hexose monophosphate shunt, except that pentose sugars are not directly formed. The two pathways are identical up to the formation of 6-phosphogluconate (see Fig. 4-4) and then diverge. In the Entner-Doudoroff pathway, no oxidative decarboxylation of 6-phosphogluconate occurs and no pentose compound is formed. For this pathway, a new 6 carbon compound intermediate (2-keto-3-deoxy6-phosphogluconate) is generated by the action of 6-phosphogluconate dehydratase (an Fe^{2+}– and glutathione-stimulated enzyme); this intermediate compound is then directly cleaved into the triose (pyruvate) and a triose-phosphate compound (glyceraldehyde-3-phosphate) by the 2-keto-3-deoxy6-phosphogluconate aldolase. The glyceraldehyde-3-phosphate is further oxidized to another pyruvate molecule by the same enzyme systems that catalyze the terminal glycolytic pathway (see Fig. 4-4).

The glycolytic pathway may be the major one existing concomitantly with the minor oxidative pentose phosphate - hexose monophosphate shunt pathway; the Entner-Doudoroff pathway also may function as a major pathway with a minor hexose monophosphate shunt. A few bacteria possess only one pathway. All cyanobacteria, *Acetobacter suboxydans*, and *A. xylinum* possess only the hexose monophosphate shunt pathway; *Pseudomonas saccharophilia* and *Z. mobilis* possess solely the Entner-Doudoroff pathway. Thus, the end products of glucose dissimilatory pathways are as follows:



The glucose dissimilation pathways used by specific microorganisms are shown in Table 4-2.

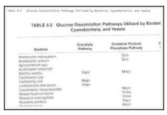


Table 4-2

Glucose Dissimilation Pathways Utilized by Bacteria, Cyanobacteria, and Yeasts.

All major pathways of glucose or hexose catabolism have several metabolic features in common. First, there are the preparatory steps by which key intermediate compounds such as the triose-PO4, glyceraldehyde-3-phosphate, and/or pyruvate are generated. The latter two compounds are almost universally required for further assimilatory or dissimilatory reactions within the cell. Second, the major source of phosphate for all reactions involving phosphorylation of glucose or other hexoses is ATP, not inorganic phosphate (Pi). Actually, chemical energy contained in ATP must be initially spent in the first step of glucose metabolism (via kinase-type enzymes) to generate glucose-6-phosphate, which initiates the reactions involving hexose catabolism. Third, NADH + H^+or NADPH + $H^+$is generated as reducing equivalents (potential energy) directly by one or more of the enzymatic reactions involved in each of these pathways.

Fermentation

Fermentation, another example of heterotrophic metabolism, requires an organic compound as a terminal electron (or hydrogen) acceptor. In fermentations, simple organic end products are formed from the anaerobic dissimilation of glucose (or some other compound). Energy (ATP) is generated through the dehydrogenation reactions that occur as glucose is broken down enzymatically. The simple organic end products formed from this incomplete biologic oxidation process also serve as final electron and hydrogen acceptors. On reduction, these organic end products are secreted into the medium as waste metabolites (usually alcohol or acid). The organic substrate compounds are incompletely oxidized by bacteria, yet yield sufficient energy for microbial growth. Glucose is the most common hexose used to study fermentation reactions.

In the late 1850s, Pasteur demonstrated that fermentation is a vital process associated with the growth of specific microorganisms, and that each type of fermentation can be defined by the principal organic end product formed (lactic acid, ethanol, acetic acid, or butyric acid). His studies on butyric acid fermentation led directly to the discovery of anaerobic microorganisms. Pasteur concluded that oxygen inhibited the microorganisms responsible for butyric acid fermentation because both bacterial mobility and butyric acid formation ceased when air was bubbled into the fermentation mixture. Pasteur also introduced the terms aerobic and anaerobic. His views on fermentation are made clear from his microbiologic studies on the production of beer (from *Etudes sur la Biere*, 1876):

> In the experiments which we have described, fermentation by yeast is seen to be the

> direct consequence of the processes of nutrition, assimilation and life, when these are carried on without the agency of free oxygen. The heat required in the accomplishment of that work must necessarily have been borrowed from the decomposition of the fermentation matter.... Fermentation by yeast appears, therefore, to be essentially connected with the property possessed by this minute cellular plant of performing its respiratory functions, somehow or other, with the oxygen existing combined in sugar.

For most microbial fermentations, glucose dissimilation occurs through the glycolytic pathway (Fig. 4-1). The simple organic compound most commonly generated is pyruvate, or a compound derived enzymatically from pyruvate, such as acetaldehyde, α-acetolactate, acetyl ~ SCoA, or lactyl ~ SCoA (Fig. 4-5). Acetaldehyde can then be reduced by NADH + H^+ to ethanol, which is excreted by the cell. The end product of lactic acid fermentation, which occurs in streptococci (e.g., *Streptococcus lactis*) and many lactobacilli (e.g., *Lactobacillus casei*, *L. pentosus*), is a single organic acid, lactic acid. Organisms that produce only lactic acid from glucose fermentation are homofermenters. Homofermentative lactic acid bacteria dissimilate glucose exclusively through the glycolytic pathway. Organisms that ferment glucose to multiple end products, such as acetic acid, ethanol, formic acid, and CO_2, are referred to as heterofermenters. Examples of heterofermentative bacteria include *Lactobacillus*, *Leuconostoc*, and *Microbacterium* species. Heterofermentative fermentations are more common among bacteria, as in the mixed-acid fermentations carried out by bacteria of the family Enterobacteriaceae (e.g., *Escherichia coli*, *Salmonella*, *Shigella*, and *Proteus* species). Many of these glucose fermenters usually produce CO_2 and H_2 with different combinations of acid end products (formate, acetate, lactate, and succinate). Other bacteria such as *Enterobacter aerogenes*, *Aeromonas*, *Serratia*, *Erwinia*, and *Bacillus* species also form CO_2 and H_2 as well as other neutral end products (ethanol, acetylmethylcarbinol [acetoin], and 2,3-butylene glycol). Many obligately anaerobic clostridia (e.g., *Clostridium saccharobutyricum*, *C. thermosaccharolyticum*) and *Butyribacterium* species ferment glucose with the production of butyrate, acetate, CO_2, and H_2, whereas other *Clostridum* species (*C. acetobutylicum* and *C. butyricum*) also form these fermentation end products plus others (butanol, acetone, isopropanol, formate, and ethanol). Similarly, the anaerobic propionic acid bacteria (*Propionibacterium* species) and the related *Veillonella* species ferment glucose to form CO_2, propionate, acetate, and succinate. In these bacteria, propionate is formed by the partial reversal of the Krebs cycle reactions and involves a CO_2fixation by pyruvate (the Wood-Werkman reaction) that forms oxaloacetate (a four-carbon intermediate). Oxaloacetate is then reduced to malate, fumarate, and succinate, which is decarboxylated to propionate. Propionate is also formed by another three-carbon pathway in *C. propionicum*, *Bacteroides ruminicola*, and *Peptostreptococcus* species, involving a lactyl ~ SCoA intermediate. The obligately aerobic acetic acid bacteria (*Acetobacter* and the related *Gluconobacter* species) can also ferment glucose, producing acetate and gluconate. Figure 4-5 summarizes the pathways by which the various major fermentation end products form from the dissimilation of glucose through the common intermediate pyruvate.

Figure 4-5

Fermentative pathways of bacteria and the major end products formed with the organism type carrying out the fermentation.

For thermodynamic reasons, bacteria that rely on fermentative process for growth cannot generate as much energy as respiring cells. In respiration, 38 ATP molecules (or approximately 380,000 cal/mole) can be generated as biologically useful energy from the complete oxidation of 1 molecule of glucose (assuming 1 NAD(P)H = 3 ATP and 1 ATP → ADP + Pi = 10,000 cal/mole). Table 4-3 shows comparable bioenergetic parameters for the lactate and ethanolic fermentations by the glycolytic pathway. Although only 2 ATP molecules are generated by this glycolytic pathway, this is apparently enough energy to permit anaerobic growth of lactic acid bacteria and the ethanolic fermenting yeast, *Saccharomyces cerevisiae*. The ATP-synthesizing reactions in the glycolytic pathway (Fig. 4-1) specifically involve the substrate phosphorylation reactions catalyzed by phosphoglycerokinase and pyruvic kinase. Although all the ATP molecules available for fermentative growth are believed to be generated by these substrate phosphorylation

reactions, some energy equivalents are also generated by proton extrusion reactions (acid liberation), which occur with intact membrane systems and involve the proton extrusion reactions of energy conservation (Fig. 4-9) as it applies to fermentative metabolism.

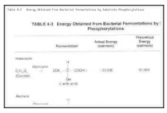


Table 4-3

Energy Obtained from Bacterial Fermentations by Substrate Phosphorylations.

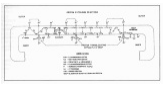


Figure 4-9

Mitchell hypotheses, a chemiosmotic model of energy transduction.

Krebs Cycle

The Krebs cycle (also called the tricarboxylic acid cycle or citic acid cycle) functions oxidatively in respiration and is the metabolic process by which pyruvate or acetyl ~ SCoA is completely decarboxylated to CO2. In bacteria, this reaction occurs through acetyl ~ SCoA, which is the first product in the oxidative decarboxylation of pyruvate by pyruvate dehydrogenase. Bioenergetically, the following overall exergonic reaction occurs:

$$CH_3{-}\overset{\overset{O}{\|}}{C}{-}COOH + 5O \xrightarrow[\text{Electron transport and oxidative phosphorylation}]{\text{Krebs cycle (via } CH_3{-}\overset{\overset{O}{\|}}{C}{-}O \sim SCoA)} 3CO_2 + 2H_2O + 15ATP\ (\simeq 150{,}000\ \text{cal/mole})$$

If 2 pyruvate molecules are obtained from the dissimilation of 1 glucose molecule, then 30 ATP molecules are generated in total. The decarboxylation of pyruvate, isocitrate, and α-ketoglutarate accounts for all CO_2 molecules generated during the respiratory process. Figure 4-6 shows the enzymatic reactions in the Krebs cycle. The chemical energy conserved by the Krebs cycle is contained in the reduced compounds generated (NADH + H^+, NADPH + H^+, and succinate). The potential energy inherent in these reduced compounds is not available as ATP until the final step of respiration (electron transport and oxidative phosphorylation) occurs.

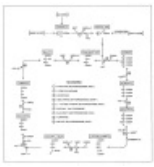


Figure 4-6

Krebs cycle (also tricarboxylic acid or citric acid cycle).

The Krebs cycle is therefore another preparatory stage in the respiratory process. If 1 molecule of pyruvate is oxidized completely to 3 molecules of CO_2, generating 15 ATP molecules, the oxidation of 1 molecule of glucose will yield as many as 38 ATP molecules, provided glucose is dissimilated by glycolysis and the Krebs cycle (further assuming that the electron transport/oxidative phosphorylation reactions are bioenergetically identical to those of eukaryotic mitochondria).

Glyoxylate Cycle

In general, the Krebs cycle functions similarly in bacteria and eukaryotic systems, but major differences are found among bacteria. One difference is that in obligate aerobes, L-malate may be oxidized directly by molecular O_2 via an electron transport chain. In other bacteria, only some Krebs cycle intermediate reactions occur because α-ketoglutarate dehydrogenase is missing.

A modification of the Krebs cycle, commonly called the glyoxylate cycle, or shunt (Fig. 4-7), which exists in some bacteria. This shunt functions similarly to the Krebs cycle but lacks many of the Krebs cycle enzyme reactions. The glyoxylate cycle is primarily an oxidative pathway in which acetyl~SCoA is generated from the oxidation, of acetate, which usually is derived from the oxidation of fatty acids. The oxidation of fatty acids to acetyl~SCoA is carried out by the β-oxidation pathway. Pyruvate oxidation is not directly involved in the glyoxylate shunt, yet this shunt yields sufficient succinate and malate, which are required for energy production (Fig. 4-7). The glyoxylate cycle also generates other precursor

compounds needed for biosynthesis (Fig. 4-7). The glyoxylate cycle was discovered as an unusual metabolic pathway during an attempt to learn how lipid (or acetate) oxidation in bacteria and plant seeds could lead to the direct biosynthesis of carbohydrates. The glyoxylate cycle converts oxaloacetate either to pyruvate and CO_2 (catalyzed by pyruvate carboxylase) or to phosphoenolpyruvate and CO_2 (catalyzed by the inosine triphosphate [ITP]-dependent phosphoenolpyruvate carboxylase kinase). Either triose compound can then be converted to glucose by reversal of the glycolytic pathway. The glyoxylate cycle is found in many bacteria, including *Azotobacter vinelandii* and particularly in organisms that grow well in media in which acetate and other Krebs cycle dicarboxylic acid intermediates are the sole carbon growth source. One primary function of the glyoxylate cycle is to replenish the tricarboxylic and dicarboxylic acid intermediates that are normally provided by the Krebs cycle. A pathway whose primary purpose is to replenish such intermediate compounds is called anaplerotic.

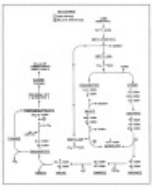


Figure 4-7

Glyoxylate shunt.

Electron Transport and Oxidative Phosphorylation

The final stage of respiration occurs through a series of oxidation-reduction electron transfer reactions that yield the energy to drive oxidative phosphorylation; this in turn produces ATP. The enzymes involved in electron transport and oxidative phosphorylation reside on the bacterial inner (cytoplasmic) membrane. This membrane is invaginated to form structures called respiratory vesicles, lamellar vesicles, or mesosomes, which function as the bacterial equivalent of the eukaryotic mitochondrial membrane.

Respiratory electron transport chains vary greatly among bacteria, and in some organisms are absent. The respiratory electron transport chain of eukaryotic mitochondria oxidizes NADH + H^+, NADPH + H^+, and succinate (as well as the coacylated fatty acids such as acetyl~SCoA). The bacterial electron transport chain also oxidizes these compounds, but it can also directly oxidize, via non-pyridine nucleotide-dependent pathways, a larger variety of reduced substrates such as lactate, malate, formate, α-glycerophosphate, H_2, and glutamate. The respiratory electron carriers in bacterial electron transport systems are more varied than in eukaryotes, and the chain is usually branched at the site(s) reacting with molecular O_2. Some electron carriers, such as nonheme iron centers and ubiquinone (coenzyme Q), are common to both the bacterial and mammalian respiratory electron transport chains. In some bacteria, the naphthoquinones or vitamin K may be found with ubiquinone. In still other bacteria, vitamin K serves in the absence of ubiquinone. In mitochondrial respiration, only one cytochrome oxidase component is found (cytochrome *a* + a_3 oxidase). In bacteria there are multiple cytochrome oxidases, including cytochromes *a*, *d*, *o*, and occasionally *a* + a_3 (Fig. 4-8)

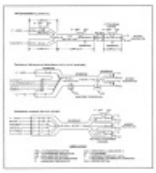


Figure 4-8

Respiratory electron transport chains.

In bacteria cytochrome oxidases usually occur as combinations of a_1: *d*: *o* and *a* + a_3:*o*. Bacteria also possess mixed-function oxidases such as cytochromes P-450 and P-420 and cytochromes *c*′ and *c*′*c*′, which also react with carbon monoxide. These diverse types of oxygen-reactive cytochromes undoubtedly have evolutionary significance. Bacteria were present before O_2 was formed; when O_2 became available as a metabolite, bacteria evolved to use it in different ways; this probably accounts for the diversity in bacterial oxygen-reactive hemoproteins.

Cytochrome oxidases in many pathogenic bacteria are studied by the bacterial oxidase reaction, which subdivides Gram-negative organisms into two major groups, oxidase positive and oxidase negative. This oxidase reaction is assayed for by using N,N,N', N'-tetramethyl-*p*-phenylenediamine oxidation (to Wurster's blue) or by using indophenol blue

synthesis (with dimethyl-*p*-phenylenediamine and α-naphthol). Oxidase-positive bacteria contain integrated (cytochrome *c* type:oxidase) complexes, the oxidase component most frequently encountered is cytochrome *o*, and occasionally *a* + a_3. The cytochrome oxidase responsible for the indophenol oxidase reaction complex was isolated from membranes of *Azotobacter vinelandii*, a bacterium with the highest respiratory rate of any known cell. The cytochrome oxidase was found to be an integrated cytochrome c_4:*o* complex, which was shown to be present in *Bacillus* species. These *Bacillus* strains are also highly oxidase positive, and most are found in morphologic group II.

Both bacterial and mammalian electron transfer systems can carry out electron transfer (oxidation) reactions with NADH + H^+, NADPH + H^+, and succinate. Energy generated from such membrane oxidations is conserved within the membrane and then transferred in a coupled manner to drive the formation of ATP. The electron transfer sequence is accomplished entirely by membrane-bound enzyme systems. As the electrons are transferred by a specific sequence of electron carriers, ATP is synthesized from ADP + inorganic phosphate (Pi) or orthophosphoric acid (H_3PO_4) (Fig. 4-8).

In respiration, the electron transfer reaction is the primary mode of generating energy; electrons (2*e*-) from a low-redox-potential compound such as NADH + H^+ are sequentially transferred to a specific flavoprotein dehydrogenase or oxidoreductase (flavin mononucleotide [FMN] type for NADH or flavin adenine dinucleotide [FAD] type for succinate); this electron pair is then transferred to a nonheme iron center (FeS) and finally to a specific ubiquinone or a naphthoquinone derivative. This transfer of electrons causes a differential chemical redox potential change so that within the membrane enough chemical energy is conserved to be transferred by a coupling mechanism to a high-energy compound (e.g., ADP + Pi → ATP). ATP molecules represent the final stable high-energy intermediate compound formed.

A similar series of redox changes also occurs between ubiquinone and cytochrome c, but with a greater differential in the oxidation-reduction potential level, which allows for another ATP synthesis step. The final electron transfer reaction occurs at the cytochrome oxidase level between reduced cyotchrome c and molecular O_2; this reaction is the terminal ATP synthesis step.

Mitchell or Proton Extrusion Hypothesis

A highly complex but attractive theory to explain energy conservation in biologic systems is the chemiosmotic coupling of oxidative and photosynthetic phosphorylations, commonly called the Mitchell hypothesis. This theory attempts to explain the conservation of free energy in this process on the basis of an osmotic potential caused by a proton concentration differential (or proton gradient) across a proton-impermeable membrane. Energy is generated by a proton extrusion reaction during membrane-bound electron transport, which in essence serve as a proton pump; energy conservation and coupling follow. This represents an obligatory “intact” membrane phenomenon. The energy thus conserved (again within the confines of the membrane and is coupled to ATP synthesis. This would occur in all biologic cells, even in the lactic acid bacteria that lack a cytochrome-dependent electron transport chain but still possesses a cytoplasmic membrane. In this hypothesis, the membrane allows for charge separation, thus forming a proton gradient that drives all bioenergization reactions. By such means, electromotive forces can be generated by oxidation-reduction reactions that can be directly coupled to ion translocations, as in the separation of H^+ and OH^- ions in electrochemical systems. Thus, an enzyme or an electron transfer carrier on a membrane that undergoes an oxidation-reduction reaction serves as a specific conductor for OH^- (or 0^{2-}), and “hydrodehydration” provides electromotive power, as it does in electrochemical cells.

The concept underlying Mitchell's hypothesis is complex, and many modifications have been proposed, but the theory's most attractive feature is that it unifies all bioenergetic conservation principles into a single concept requiring an intact membrane vesicle to function properly. Figure 4-9 shows how the Mitchell hypothesis might be used to explain energy generation, conservation, and transfer by a coupling process. The least satisfying aspect of the chemiosmotic hypothesis is the lack of understanding of how chemical energy is actually conserved within the membrane and how it is transmitted by coupling for ATP synthesis.

Bacterial Photosynthesis

Many prokaryotes (bacteria and cyanobacteria) possess phototrophic modes of metabolism (Table 4-1) . The types of photosynthesis in the two groups of prokaryotes differ mainly in the type of compound that serves as the hydrogen donor in the reduction of CO_2 to glucose (Table 4-1). Phototrophic organisms differ from heterotrophic organisms in that they utilize the glucose synthesized intracellularly for biosynthetic purposes (as in starch synthesis) or for energy production, which usually occurs through cellular respiration.

Unlike phototrophs, heterotrophs require glucose (or some other preformed organic compound) that is directly supplied as a substrate from an exogenous source. Heterotrophs cannot synthesize large concentrations of glucose from $CO_2$by specifically using H_2O or (H_2S) as a hydrogen source and sunlight as energy. Plant metabolism is a classic example of photolithotrophic metabolism: plants need CO_2 and sunlight; H_2O must be provided as a hydrogen source and usually NO_3^- is the nitrogen source for protein synthesis. Organic nitrogen, supplied as fertilizer, is converted to NO_3^- in all soils by bacteria via the process of ammonification and nitrification. Although plant cells are phototrophic, they also exhibit a heterotrophic mode of metabolism in that they respire. For example, plants use classic respiration to catabolize glucose that is generated photosynthetically. Mitochondria as well as the soluble enzymes of the glycolytic pathway are required for glucose dissimilation, and these enzymes are also found in all plant cells. The soluble Calvin cycle enzymes, which are required for glucose synthesis during photosynthesis, are also found in plant cells. It is not possible to feed a plant by pouring a glucose solution on it, but water supplied to a plant will be “photolysed” by chloroplasts in the presence of light; the hydrogen(s) generated from H_2O is used by Photosystems I and II (PSI and PSII) to reduce $NADP^+$ to NADPH + H^+. With the ATP generated by PSI and PSII, these reduced pyridine nucleotides, CO_2 is reduced intracellularly to glucose. This metabolic process is carried out in an integrated manner by Photosystems I and II (“Z” scheme) and by the Calvin cycle pathway. A new photosynthetic, and nitrogen fixing bacterium, *Heliobacterium chlorum*, staining Gram positive was isolated, characterized, and found to contain a new type of chlorophyll, i.e., bacteriochlorophyll ‘g’. 16S r-RNA sequence analyses showed this organism to be phylogenetically related to members of the family *Bacillaceae*, although all currently known phototrophes are Gram negative (see Table 4.4). A few *Heliobacteriium* strains did show the presence of endospores. Another unusual phototrophe is the Gram negative *Halobacterium halobium* (now named *Halobacterium salinarium*), an archaebacterium growing best at 30°C in 4.0–5.0 M (or 25%, w/v) NaCl. This bacterium is a facultative phototrophe having a respiratory mode; it also possesses a purple membrane within which bacteriorhodopsin serves as the active photosynthetic pigment. This purple membranae possesses a light driven proton translocation pump which mediates photosynthetic ATP synthesis via a proton extrusion reaction (see Mitchell Hypothesis). Table 4-4 summarizes the characteristics of known photosynthetic bacteria.

Table 4-4

Characteristics Commonly Exhibited by Phototrophic Bacteria[a].

Autotrophy

Bacteria that grow solely at the expense of inorganic compounds (mineral ions), without using sunlight as an energy source, are called autotrophs, chemotrophs, chemoautotrophs, or chemolithotrophs. Like photosynthetic organisms, all autotrophs use CO_2 as a carbon source for growth; their nitrogen comes from inorganic compounds such as NH_3, NO_3^-, or N_2 (Table 4-1). Interestingly, the energy source for such organisms is the oxidation of specific inorganic compounds. Which inorganic compound is oxidized depends on the bacteria in question (Table 4-5). Many autotrophs will not grow on media that contain organic matter, even agar.

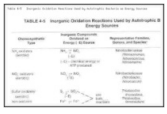


Table 4-5

Inorganic Oxidation Reactions Used by Autotrophic Bacteria as Energy Sources.

Also found among the autotrophic microorganisms are the sulfur-oxidizing or sulfur-compound-oxidizing bacteria, which seldom exhibit a strictly autotrophic mode of metabolism like the obligate nitrifying bacteria (see discussion of nitrogen cycle below). The

representative sulfur compounds oxidized by such bacteria are H_2S, S_2, and S_2O_3. Among the sulfur bacteria are two very interesting organisms; *Thiobacillus ferrooxidans*, which gets its energy for autotrophic growth by oxidizing elemental sulfur or ferrous iron, and *T. denitrificans*, which gets its energy by oxidizing S_2O_3 anaerobically, using NO_3^- as the sole terminal electron acceptor. T denitrificans reduces NO_3 to molecular N_2, which is liberated as a gas; this biologic process is called denitrification.

All autotrophic bacteria must assimilate CO_2, which is reduced to glucose from which organic cellular matter is synthesized. The energy for this biosynthetic process is derived from the oxidation of inorganic compounds discussed in the previous paragraph. Note that all autotrophic and phototrophic bacteria possess essentially the same organic cellular constituents found in heterotrophic bacteria; from a nutritional viewpoint, however, the autotrophic mode of metabolism is unique, occurring only in bacteria.

Anerobic Respiration

Some bacteria exhibit a unique mode of respiration called anaerobic respiration. These heterotrophic bacteria that will not grow anaerobically unless a specific chemical component, which serves as a terminal electron acceptor, is added to the medium. Among these electron acceptors are NO_3^-, SO_4^{2-}, the organic compound fumarate, and CO_2. Bacteria requiring one of these compounds for anaerobic growth are said to be anaerobic respirers.

A large group of anaerobic respirers are the nitrate reducers (Table 4-6). The nitrate reducers are predominantly heterotrophic bacteria that possess a complex electron transport system(s) allowing the NO_3^- ion to serve anaerobically as a terminal acceptor of electrons

$(NO_3^- \xrightarrow{2e^-} NO_2^-;\ NO_3^- \xrightarrow{5e^-} N_2;\ \text{or}\ NO_3^- \xrightarrow{8e^-} NH_3)$

. The organic compounds that serve as specific electron donors for these three known nitrate reduction processes are shown in Table 4-6. The nitrate reductase activity is common in bacteria and is routinely used in the simple nitrate reductase test to identify bacteria (see *Bergey's Manual of Deterininative Bacteriology*, 8th ed.).

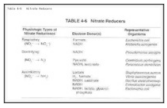


Table 4-6

Nitrate Reducers.

$$4AH_2 + HNO_3 \xrightarrow{\text{Nitrate reduction}} 4A + NH_3 + 3H_2O + \text{energy}$$

(AH_2 = organic substrate, which serves as electron donor)

A second group of anaerobic respirers, the sulfate reduoers, utilizes SO_4^{2-} ion in similar fashion

$(SO_4^{2-} \xrightarrow{8e^-} H_2S)$:

$$4AH_2 + H_2SO_4 \xrightarrow{\text{Sulfate reduction}} 4A + H_2S + 4H_2O + \text{energy}$$

The third group, the fumarate respirers, are anaerobic bacteria that require exogenous HOOC — CH = CH — COOH for growth. Fumarate is reduced to succinate (HOOC — CH_2 — CH_2 — COOH), which is secreted as a by-product.

$$AH_2 + HOOC{=}\overset{H}{\overset{\|}{C}} - \overset{H}{\overset{\|}{C}}{-}COOH \xrightarrow{\text{Fumarate reduction}} A + HOOC - CH_2 - CH_2 - COOH + \text{energy}$$

Organisms of still another specialized group of anaerobic respirers, the methanogens, produce methane gas $CO_2 \xrightarrow{8e^-} CH_4$ as a metabolic end product of microbial growth. H_2 gas is the growth substrate; CO_2 is the terminal electron acceptor.

$$4H_2 + CO_2 \xrightarrow{CO_2\ \text{reduction}} CH_4 + 2H_2O + \text{energy}$$

The methanogens are among the most anaerobic bacteria known, being very sensitive to

small concentrations of molecular O_2. They are also archaebacteria, which typically live in unusual and deleterious environments.

All of the above anaerobic respirers obtain chemical energy for growth by using these anaerobic energy-yielding oxidation reactions.

The Nitrogen Cycle

Nowhere can the total metabolic potential of bacteria and their diverse chemical-transforming capabilities be more fully appreciated than in the geochemical cycling of the element nitrogen. All the basic chemical elements (S, O, P, C, and H) required to sustain living organisms have geochemical cycles similar to the nitrogen cycle.

The nitrogen cycle is an ideal demonstration of the ecologic interdependence of bacteria, plants, and animals. Nitrogen is recycled when organisms use one form of nitrogen for growth and excrete another nitrogenous compound as a waste product. This waste product is in turn utilized by another type of organism as a growth or energy substrate. Figure 4-10 shows the nitrogen cycle.

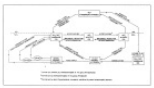


Figure 4-10

The nitrogen cycle.

When the specific breakdown of organic nitrogenous compounds occurs, that is, when proteins are degraded to amino acids (proteolysis) and then to inorganic NH_3, by heterotrophic bacteria, the process is called ammonification. This is an essential step in the nitrogen cycle. At death, the organic constituents of the tissues and cells decompose biologically to inorganic constituents by a process called mineralization; these inorganic end products can then serve as nutrients for other life forms. The NH_3 liberated in turn serves as a utilizable nitrogen source for many other bacteria. The breakdown of feces and urine also occurs by ammonification.

The other important biologic processes in the nitrogen cycle include nitrification (the conversion of NH_3 to $NO_3$by autotrophes in the soil; denitrification (the anaerobic conversion of NO_3 to N_2 gas) carried out by many heterotrophs); and nitrogen fixation ($N_2$to NH_3, and cell protein). The latter is a very specialized prokaryotic process called diazotrophy, carried out by both free-living bacteria (such as *Azotobacter*, *Derxia*, *Beijeringeia*, and *Azomona* species) and symbionts (such as *Rhizobium* species) in conjunction with legume plants (such as soybeans, peas, clover, and bluebonnets). All plant life relies heavily on NO_3^- as a nitrogen source, and most animal life relies on plant life for nutrients.

References

1. Buchanan RE, Cibbons NE (eds): Bergey's Manual of Determinative Bacteriology. 8th Ed. Williams & Wilkins, Baltimore, 1974 .
2. Green DE. A critique of the chemosmotic model of energy coupling. Proc Natl Acad Sci USA. 1981;78:2249. [PMC free article: PMC319320] [PubMed: 6264470]
3. Haddock BA, Hamilton WA (eds): Microbial energetics. 27th Symposium of the Society of General Microbiology. Cambridge University Press, Cambridge, 1977 .
4. Hempfling WP: Microbial Respiration. Benchman Papers in Microbiology no. 13.
5. Downden, Hutchinson and Ross, Stroudsburg, PA, 1979 .
6. Hill R: The biochemists' green mansions: the photosynthetic electron-transport chain in plants. In Campbell PN, Greville CD (eds): Essays in Biochemistry. Vol.1. Academic Press, New York, 1965 . [PubMed: 4387015]
7. Jurtshuk P Jr, Liu JK. Cytochrome oxidase and analyses of *Bacillus* strains: existence of oxidase-positive species. Int J Syst Bacterol. 1983;33:887.
8. Jurtshuk P Jr, Mueller TJ, Acord WC. Bacterial terminal oxidases. Crit Rev Microbiol. 1975;3:359. [PubMed: 166799]
9. Jurtshuk P Jr, Mueller TJ, Wong TY. Isolation and purification of the cytochrome oxidase of *Azotobacter vinelandii*. Biochim Biophys Acta. 1981;637:374. [PubMed: 6271199]
10. Jurtshuk P, Jr, Yang TY: Oxygen reactive hemoprotein components in bacterial respiratory systems. In Knowles CJ (ed): Diversity of Bacterial Respiratory Systems. Vol. 1. CRC Press, Boca Raton, FL, 1980 .

11. Kamp AF, La Riviere JWM, Verhoeven W (eds): Jan Albert Kluyver: His Life and Work. Interscience, New York, 1959 .
12. Kluyver JA, Van Niel CB: The microbe's contribution to biology. Harvard University Press, Cambridge, MA, 1956 .
13. Kornberg HL: The role and maintenance of the tricarboxylic acid cycle in *Escherichia coli*. In Goodwin TW (ed): British Biochemistry Past and Present. Biochemistry Society Symposium no. 30. Academic Press, London, 1970 . [PubMed: 4322317]
14. Lemberg R, Barrett J: Bacterial cytochromes and cytochrome oxidases. In Lemberg R, Barrett J: Cytochromes. Academic Press, New York, 1973 .
15. Mandelstam J, McQuillen K, Dawes I (eds): Biochemistry of Bacterial Growth. 3rd Ed. Blackwell, Oxford, 1982 .
16. O'Leary WM: The chemistry and metabolism of microbial lipids. World Publishing Co, Cleveland, 1967 .
17. Schlegel HG, Bowier B (eds): Autotrophic Bacteria. Science Tech, Madison, Wl, 1989 .
18. Slepecky RA, Leadbetter ER: Ecology and relationships of endospore-forming bacteria: Changing perspectives. In Piggot P, Moran Jr, CP and Youngman P (eds). Regulation of Bacterial Differentiation. Am Soc Microbiol Press, 1994 .
19. Thauer RK, Jungermann K, Decker K. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev. 1977;41:100. [PMC free article: PMC413997] [PubMed: 860983]
20. Thimann KV: The Life of Bacteria. 2nd Ed. Macmillan, New York, 1966 .

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