KAPLAN

NDB Part I Review Notes

Volume One

MICROBIOLOGY & IMMUNOLOGY

PATHOLOGY

BIOCHEMISTRY

MOLECULAR BIOLOGY

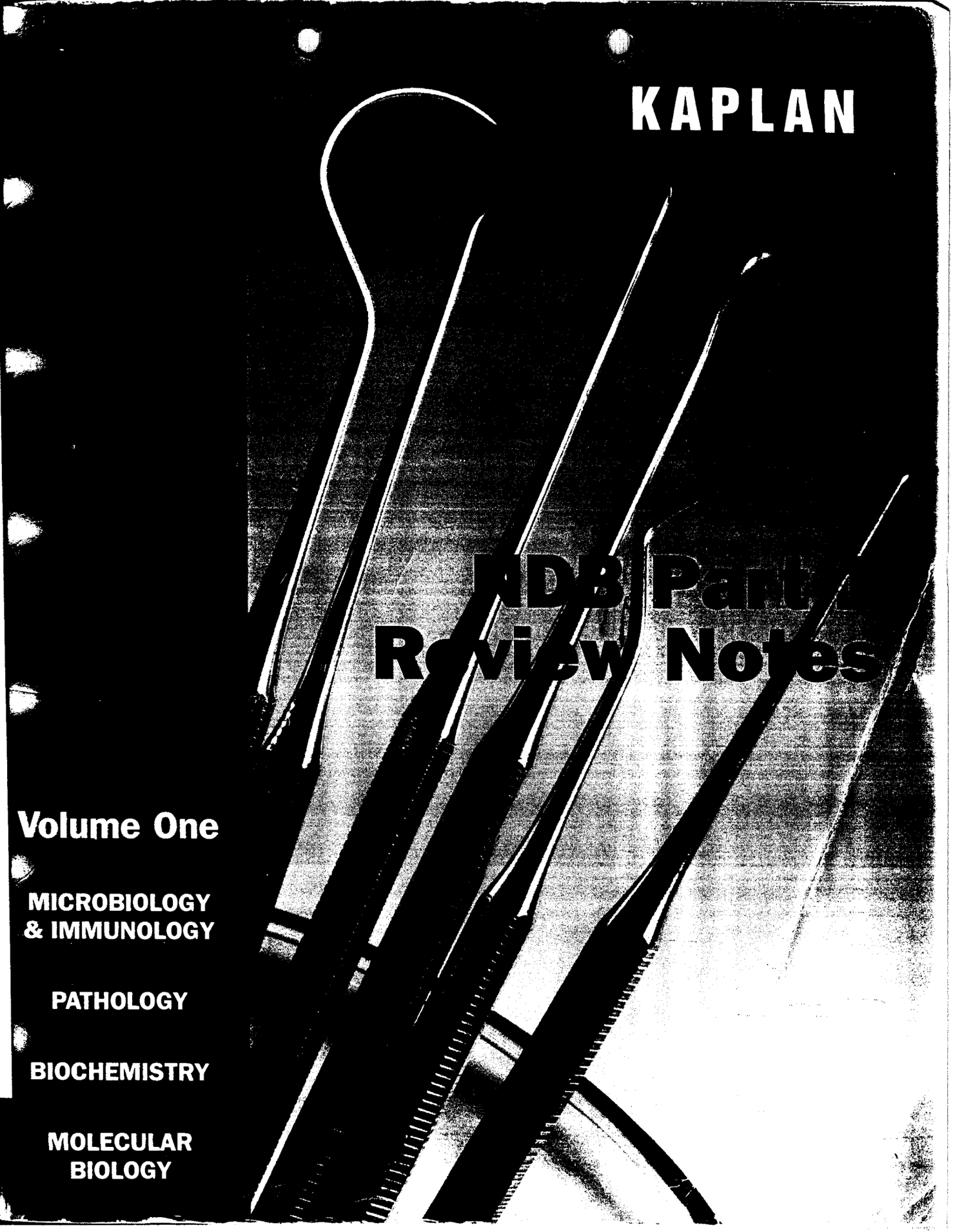

TABLE OF CONTENTS

PART III: BIOCHEMISTRY

PART IV: MOLECULAR BIOLOGY

PART I

MICROBIOLOGY AND IMMUNOLOGY

Introduction

The human body plays host to a vast number of microorganisms. This brief introductory chapter describes the normal microbial flora that colonize different anatomical sites as well as the variety of factors that can confer virulence to selected microorganisms.

NORMAL MICROBIAL FLORA

A. **Properties.** Normal microbial flora describes the population of microorganisms that usually reside in the body. The microbiological flora can be defined as either **"resident flora,"** a relatively-fixed population that will repopulate if disturbed, or **"transient flora"** that are derived from the local environment. These microbes usually reside in the body without invasion and can even prevent infection by more pathogenic organisms, a phenomenon known as **bacterial interference.** The flora have commensal functions such as vitamin K synthesis. However, they may cause invasive disease in immunocompromised hosts or if displaced from their normal area.

B. **Location.** Microbial flora differ in composition depending on their anatomical locations and microenvironments. The distribution of normal microbial flora is summarized in Table 1-1.

NOTE

Normal flora prevent pathogen colonization by occupying the receptor sites.

MICROBIAL VIRULENCE FACTORS

Microbial virulence factors are gene products required for a microbial pathogen to establish itself in the host. These gene products are located on the bacterial chromosome, or on mobile genetic elements, such as plasmids or transposons. **Primary pathogens** express virulence factors that allow them to cause disease in the normal host. **Opportunistic pathogens** are environmental organisms or normal flora that lack the means to overcome normal host defense

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mechanisms. They cause disease only when the normal host defenses are breached or deficient. Virulence factors can be divided into several categories.

Location	Major organisms of the normal flora
Skin	*Propionibacterium acnes, Staphlococcus epidermidis,* diptheroids; transient colonization by *Staphlococcus aureus*
Oral cavity	Viridans *Streptococci, Branhamella* species, *Prevotella melaninogenicus, Actinomyces* species, *Peptostreptococcus* species, other anaerobes
Nasopharynx	Oral organisms; transient colonization by *S. pneumoniae, Haemophilus* species, *N. meningitidis*
Stomach	Rapidly becomes sterile
Small intestine	Scant
Colon	*Bacteroides* species, *Clostridium* species, *Fusobacterium* species, *E. coli, Proteus* species, *Pseudomonas aeruginosa, Enterococcus* species, other bacteria and yeasts
Vagina	Childbearing years: *Lactobacillus* species, yeasts, *Streptococcus* species Prepuberty/Postmenopause: colonic and skin flora

Table 1-1. Major species of microbial flora found in different anatomical locations.

A. **Enzyme production** can be of several types depending on the needs of the organism, its requirements for survival, and the local environment.
   1. **Hyaluronidase** breaks down hyaluronic acid to aid in the digestion of tissue.
   2. **Protease** digests proteins to enhance the spread of infections.
   3. **Coagulase** allows coagulation of fibrinogen to clot plasma.
   4. **Collagenase** breaks down collagen (connective tissues).

B. **Toxins**
   1. **Exotoxins** are heat-labile proteins with specific enzymatic activities produced by many **Gram-positive** and **Gram-negative** organisms. Exotoxins are released extracellularly and are often the sole cause of disease.
      a. Some toxins have several domains with discrete biological functions that confer maximal toxicity. An example is **A-B exotoxin,** where the B subunit binds to host tissue cell glycoproteins and the A subunit enzymatically attacks a susceptible target.
      b. Many toxins are **ADP-ribosylating toxins,** as outlined below in Table 1-2.
   2. **Endotoxin** is the heat-stable **lipopolysaccharide** moiety found in the outer membrane of **Gram-negative organisms. When**

NOTE:

NDB commonly asks about normal oral flora. The most common are the viridans streptococci (gram-positive, facultatively anaerobic cocci). Strep mutans in particular is the major cause of caries.

IN A NUTSHELL

Exotoxin	***Endotoxin***
Protein	*Lipopolysaccharide*
Gram⁺ and Gram⁻	*Gram⁻*
Released extracellularly	*Part of outer membrane; not secreted*
Heat labile	*Heat stabile*

released by cell lysis, the **lipid A portion** of lipopolysaccharide can induce septic shock characterized by fever, acidosis, hypotension, complement consumption, and disseminated intravascular coagulation (DIC).

C. **Surface components** may protect the organism from immune responses such as phagocytosis or aid in tissue invasion. For example, the **polysaccharide capsules** of *H. influenzae* type b and the acidic polysaccharide capsule of *Streptococcus pneumoniae* interfere with phagocytosis. Other surface proteins, such as adhesins or filamentous appendages (fimbriae, pili), are involved in adherence of invading microorganisms to cells of the host.

Toxin	Structure	Substrate	Effects
Diphtheria	A-B	Elongation factor II	↓ Protein synthesis
Pseudomonas exotoxin A			
Cholera *E. coli* enterotoxin	$A\text{-}B_5$	Adenylate cyclase regulatory protein	↑ Adenylate cyclase
Pertussis			
Shiga (dysentery)		60S ribosome	↓ Protein synthesis
Tetanus/botulinum		Synaptobrevin	Nerve dysfunction

Table 1-2. Properties of ADP-ribosylating exotoxins.

Bacteriology Overview

This chapter reviews the essential features of bacterial structure with an emphasis on the differences between Gram-positive and Gram-negative organisms. Also discussed are important concepts relating to bacterial growth, metabolism, sporulation, and the methods by which bacteria transfer genetic information.

CLASSIFICATION AND IDENTIFICATION OF BACTERIA

A. **General properties** can differentiate prokaryotic (single-celled) organisms from higher eukaryotic organisms. The most distinguishable features include:

1. Prokaryotes have **70S ribosomes,** while eukaryotes have 80S ribosomes. This difference allows some classes of antibiotics to specifically target prokaryotic protein biosynthesis.
2. Prokaryotes have a **naked, single, circular chromosome** of double-stranded DNA that replicates bidirectionally. There is **no true nucleus.** Prokaryotes lack a nuclear membrane and their DNA does not have basic proteins (histones) associated with it.
3. Prokaryotes **lack membrane-bound organelles** such as mitochondria.
4. The **cell wall** of most bacteria is a unique, rigid, peptidoglycan layer that allows the characteristic Gram-positive and Gram-negative staining of bacteria. **Gram stain** (positive vs. negative) is based on biochemical retention of dyes by the cell's outermost layer; Gram-positives have a larger amount of peptidoglycan. **Acid-fast staining** is based on the ability to resist acid decolorization due to a high content of waxes in the cell wall. Mycobacteria species are acid fast and Nocardia species are partially acid fast.

NOTE

The mycoplasmas (including ureaplasma) are the only bacteria that do not have cell walls. Chlamydia have cell walls that lack muramic acid. Both mycoplasma and chlamydia are therefore resistant to beta-lactam antibiotics.

4. **Flagella** are responsible for bacterial motility.
5. **Pili or fimbriae** are used for attachment to host cells and are required for conjugation in Gram-negative bacteria. These structures convey adhesive properties to bacteria (adhesin).

B. **Classification of bacteria** is defined arbitrarily by the biochemical characteristics and/or phenotypic features that allow look-alike organisms to be differentiated from each other. The genus and species of an isolate can be determined by morphology and Gram stain, and by biochemical and nutritional traits. Some specific examples include:

1. **Biochemical characteristics** such as substrate specificity (e.g., *B. pertussis* grows specifically on Bordet-Gengou agar), the ability to ferment specific sugars (e.g., *E. coli* are lactose fermentors), and the production of unique metabolic products (e.g., *Mycobacterium tuberculosis* produces niacin).
2. **Serologic reactivity**—the identification with specific antibodies in diagnostic immunoassays.
3. **Bacteriophage typing** is useful epidemiologically in tracing the source of epidemics.
4. **Animal pathogenicity**
5. **Antibiotic sensitivity**

BACTERIAL STRUCTURE

A. The **cell envelope** is defined as all layers that enclose the cytosol of a bacterium. It is the main structural feature that differentiates Gram-positive from Gram-negative bacteria. Gram-positive bacteria have a smooth or finely patterned surface consisting of the cytoplasmic membrane, peptidoglycan layer, and sometimes, an outer capsule. Gram-negative bacteria have a complex cell envelope consisting of a cytoplasmic membrane (inner membrane), **periplasmic space** containing peptidoglycan, an **outer membrane,** and sometimes, a capsule.

1. **Capsule production** is usually correlated with virulence since capsules are antiphagocytic. Most capsules are carbohydrate in nature.
2. The **slime layer** is easily washed off and is less adherent than capsules.
3. The **bacterial cell wall** is a structure unique to prokaryotes and is a major site of antibiotic attack (e.g., penicillin blocks peptidoglycan synthesis). .

a. **Gram-positive bacteria** are composed of a thick layer of peptidoglycan, **lipoteichoic acids,** polysaccharides, and sometimes, **teichoic acid.** The surface proteins bind to extracellular material.

b. **Gram-negative bacteria** have a trilayered outer membrane anchored to the cell membrane by **lipoprotein. Endotoxin** (lipopolysaccharide, somatic O antigen, and core polysaccharide) in the outer membrane is unique to Gram-negative bacteria. **Protein porin channels** allow the flow of extracellular material through the cell's outer membrane.

4. The **periplasm** is the space between the plasma membrane and the outer membrane. It contains proteins, peptidoglycan, hydrolytic enzymes, and plasmid-controlled penicillinases. The periplasmic space is important in osmoregulation of the cell.

B. **Plasma (cell) membrane** functions primarily as an osmotic barrier. Its composition is 60-70% protein, 30-40% lipid, and small amounts of carbohydrate. If present, the **bacterial electron transport chain** is located within the cytoplasmic membrane. Structures associated with the plasma membrane are membrane polyribosome-DNA aggregates and **mesosomes.** Mesosomes are convoluted structures of cell membrane important in cell division.

C. **Cytoplasmic structures**

1. The **nucleoid region** of bacteria consists of a circular chromosome of double-stranded DNA that lacks introns, histones, and a nuclear membrane. In contrast, eukaryotic nuclei contain all of the above.

2. **Ribosomes** consist of 70% RNA and 30% protein. 70S ribosomes (monomers) are attached to messenger RNA. The 70S complex can be broken down into subunits of 30S and 50S.

3. **Polyamines** (e.g., putrescine) are located mainly in ribosomes and serve to prevent dissociation of the 70S ribosome.

4. **Cytoplasmic granules** function to accumulate food reserves such as glycogen, lipids in the form of poly-β-hydroxybutyrate, and phosphate in the form of volutin granules.

5. **Spores** (endospores) are found in Bacillus and Clostridium species. Spores promote the survival of the organism under adverse environmental conditions since they **resist heat and drying.** Spores are highly dehydrated and refractile. They convert into vegetative cells via germination when conditions of the environment are more favorable.

Tables 2-1, 2-2, and 2-3 summarize the important features of bacterial structure and the key differences between Gram-positive and Gram-negative organisms.

IN A NUTSHELL

Gram-positives:
- *Thick peptidoglycan cell wall overlays cell membrane*
- *No outer membrane*
- *Cell wall contains lipoteichoic acids and some have teichoic acid (virulence factor)*

Gram-negatives:
- *Thin peptidoglycan cell wall overlays cell membrane*
- *Also have an outer membrane*
- *Space between cell membrane and outer membrane includes peptidoglycan and is called the periplasmic space*
- *Lipoprotein in the periplasmic space connects the outer membrane to the cell membrane*
- *Lipopolysaccharide (LPS) is a component of the outer membrane:*
  Lipid A = toxic portion

NOTE

Because the spores of Bacillus sp. are resistant to the most extreme environmental conditions, they are used to check the effectiveness of autoclaves in instrument sterilization.

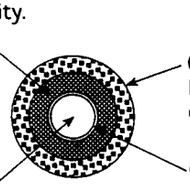
- Nucleoid – single, circular, ds DNA chromosome; not called a nucleus because it lacks a membrane
- No sterols in membrane (exception: Mycoplasma)
- 70S ribosomes
- No membrane-bound organelles
- Rigid cell wall of peptidoglycan [Note: cell envelope = all layers that encircle and retain cytoplasmic contents; cell wall = peptidoglycan (PDG)]
  - N-acetylglucosamine
  - N-acetylmuramic acid with tetrapeptide in a β(1,4) linkage (lysozyme breaks this linkage)
  - D and L alternating amino acids, tetrapeptide cross-bridges linked by transpeptidase (All of the above contribute to PDG strength)
  - Function – shape, barrier, sieve, prevents lysis (bacteria are subjected to 2-5× the pressure that tissues face)
- Amino acids found in peptidoglycan
  - D-glutamic acid
    D-alanine
  - L-alanine (primarily Gram-negatives)
  - Diaminopimelic acid
  - L-lysine (primarily Gram-positives)
- Cytoplasmic membrane:
  - Structure – normal membrane
  - Function – osmotic barrier; active transport of nutrients via permeases; DNA attachment site for nucleoid and plasmids; iron uptake via siderophores; protein export, since Golgi and ER are lacking; site of electron transport chain, since mitochondria are lacking
- Mesosome
  - Structure – membrane invagination – more prominent in Gram-positive (some say only in Gram-positive)
  - Function – site of DNA replication, cell division
- Introns absent
- Transcription directly coupled to translation
- Polygenic mRNAs
- Initiator tRNA is formyl methionyl-tRNA instead of methionyl-tRNA

Table 2-1. Features common to all prokaryotes.

- **Unique metabolic capacities**
  - Obligate anaerobes
  - Fix N_2
- **Capsule**
  - Structure – acidic polysaccharides of 2-3 sugars characteristic of the organism
  - Function – antiphagocytic
  - Note: a capsule has a well-defined border, a slime layer just trails off into the media
- **Flagella:**
  - Structure – single proteinaceous helical filament of globular protein flagellin. Adds at the tip.
  - 3 parts: basal body (anchored in cell membrane), filament, and hook. Counterclockwise rotation = coordinated movement towards a substance. Clockwise rotation = disorganized tumble away from a source.
  - Function – locomotion, chemotaxis

  2 variations:
  - Peritrichous – all over, e.g., Enterobacteriacae
  - Polar – at one end, e.g., Pseudomonas
  - Other polar nomenclature: lophotrichous – tuft of several flagella; amphitrichous – both poles have flagella.
- **Glycocalyx structure:**
  - Structure – adhesive polymer film containing polysaccharides
  - Function – inhibits phagocytosis and antibody access/adhesion
- **Storage granules:**
  - Poly β hydroxybutarate = lipid
  - Glycogen = starch
  - Polymetaphosphate (volutin)-phosphate
- Electron transport chains are located in the cytoplasmic membrane
- **Spores or endospores (in some medically-important Gram-positives)**
  - Cryptobiotic = dormant , no metabolic activity. Resistant to heat, dessication, freezing, etc.
  - The inner membrane is also called the germ cell membrane.
  - Coat has 80% of protein

Cortex – Unique peptidoglycan in 2 layers: outer layer has few cross-links, rapid autolysis; inner layer has more cross-links and osmotic stability.



Coat – keratin-like protein layers with many cross-linking disulfide bonds

Germ cell membrane

Core -Dipicolinic acid, Ca^{2+}, chromosome, protein, and materials for resuming growth exist here

- Coat proteins can't be solublized unless the disulfide groups are reduced – this cross-linked layer is responsible for the resistance of spores to chemicals.
- Ca^{2+} and dipicolinic acid are chelated in equal amounts in the core – helps keep the water content down by displacing water when intercalation occurs with the DNA.
- Seen in aerobic Bacillus and anaerobic Clostridium species.

Table 2-2. Features found in some prokaryotes.

Gram-positive	Gram-negative
• **Cell envelope:** – PDG 100-800 Å – Plasma (cytoplasmic) membrane • **Lipoteichoic acid:** – Structure – glycerol or ribitol residues with phosphodiester links and substitutions anchored to glycolipid in membrane – (so it goes all the way through the PDG) – Function – anchoring and adhesion • Proteins excreted outside cell	• **Cell envelope:** – Outer membrane – 60-180 Å-sieve – PDG 20-30 Å – Plasma membrane • Periplasmic space – contains PDG, enzymes, PBP, binding proteins that soak up sugars and amino acids, etc. • Membrane-derived oligosaccharide (MDO): – Structure – glucan polymer secreted into periplasmic space – Function – regulates osmolality of periplasmic space – adjusts viscosity to avoid lysis • Lipopolysaccharide (endotoxin): – Structure and functions – Lipid A, which is responsible for endotoxin manifestations (hypotension and DIC), and a long oligosaccharide side chain that is group-specific and hydrophilic (this helps to exclude hydrophobic compounds) • Porins: – Structure – protein trimer bound noncovalently to PDG – Function – restricts entry of > 800 MW molecules • Adhesins and/or evasins – Function: an adhesin is a gene product promoting attachment and colonization. An evasin is a gene product promoting evasion of host defenses. • Lipoprotein: – Function – covalently anchors outer membrane onto PDG; also loosely linked to LPS • **Fimbriae or pili:** – Structure – filament of pilin, helical arrangement, addition at base **Sex pili:** – Genetic exchange (plasmid-determined) – Only see a few per cell (3-4) **Common pili or fimbriae:** – Adhere to host cells – Several 100 per cell – Almost all Gram-negatives have them; some Gram-positives as well – Can also act as evasins or aggresins

Table 2-3. Features found in Gram-positive and Gram-negative bacteria.

BACTERIAL GROWTH

How rapidly an infection can progress before host defenses respond can determine the severity of disease. In a closed system, growth is dependent upon the availability of nutrients, the external environment (e.g., temperature), and the growth rate of the specific species. Figure 2-1 depicts a typical bacterial growth curve.

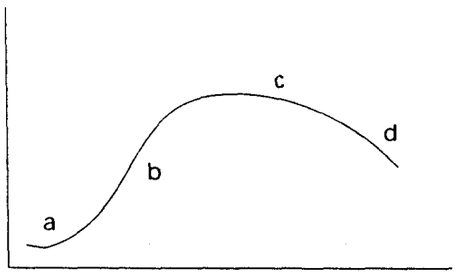


Figure 2-1. Growth curve in a closed system. a = lag phase; b = exponential or log phase; 3 = stationary phase; d = phase of decline.

A. **Bacterial growth in a closed system**

1. The **lag phase** is a period of no growth when the organisms are adapting to a new environment.
2. The **exponential or log phase** describes the steady state of growth, typically at the organism's fastest rate. It continues until the nutrients are depleted or toxic waste products accumulate. Many antibiotics, especially those that target cell wall synthesis, are maximally effective during this phase.
3. The **stationary phase** occurs when nutrients are exhausted or toxins accumulate. Cell number is stable (cell loss = cell formation). Different outcomes can occur depending on the species of bacteria. Some bacteria stop growing, but remain viable for long periods of time. Some organisms cannot maintain a viable, nongrowing state. When they reach the stationary phase, they immediately start to die. Other organisms, if conditions do not improve, start forming spores.
4. The **phase of decline** is observed when the death rate increases, due to cell starvation or sensitivity to toxins.

SURVIVAL IN OXYGEN

Survival in oxygen is an important parameter used to classify bacteria. All bacteria produce the superoxide ion (O_2-) in the presence of oxygen. Three enzymes are important to detoxify this ion. **Superoxide dismutase** converts the superoxide ion to hydrogen peroxide. **Catalase** or **peroxidase** then metabolizes the hydrogen peroxide to water and oxygen. **Obligate anaerobes** (strict anaerobes) lack these enzymes or have such low levels that an oxygen environment is toxic for them. **Facultative organisms** grow with or without oxygen. Whether they ferment or respire is an independent issue.

NOTE

Oxygen is toxic to obligate anaerobic organisms because they lack superoxide dismutase.

ENERGY PRODUCTION

Energy production requires a source of carbon. Some bacteria are very versatile, requiring only a few essential nutrients. Many pathogenic bacteria have become so host adapted that they have lost much of their metabolic machinery. They have many additional requirements and are termed **fastidious bacteria.**

A. **Siderophores** are iron- (Fe^{3+}) chelating compounds that are essential for growth of many pathogenic bacteria. The siderophore is a low-molecular weight material such as a catechol that is excreted from the cell. This molecule binds iron and is then bound to a cell wall or outer membrane protein and transported into the cytosol of the cell, where it is utilized in energy metabolism.

B. **Mechanisms of energy production**

1. **Fermentation** is the **anaerobic** degradation of glucose to obtain ATP.
   a. It is much less efficient than respiration for generating energy. It can be used by both obligate anaerobes and facultative organisms. End products of fermentation differ depending upon the organism, and are often useful in identification.
   b. Most obligate anaerobes and all Streptococcus species use fermentation. Streptococcus species can only ferment because they cannot make cytochromes or catalase. However, they do have superoxide dismutase and peroxidase, so they can survive in an oxygen environment.
2. **Respiration** completely oxidizes organic fuels, and requires an **electron transport chain** to drive the synthesis of ATP.
   a. Respiration produces almost twenty times as much ATP as fermentation. It requires a terminal electron acceptor. The usual electron acceptor is oxygen; however, alternate electron acceptors, such as nitrate and fumarate, are used by some organisms.
   b. Given a choice, bacteria will opt for respiration over fermentation. However, bacteria differ in their intrinsic ability to use fermentation or respiration.
      (1) Strict or **obligate aerobes** respire only, and must use oxygen as a terminal electron acceptor. *Mycobacterium tuberculosis* is a good example of an obligate aerobe.
      (2) Some bacteria ferment only.
      (3) The majority of bacteria use the most versatile strategy, fermentation or respiration, depending upon the conditions.

SPORULATION

A. A **spore** is a dormant structure capable of surviving prolonged periods of unfavorable environmental forces. Spores are capable of re-establishing the vegetative stage of growth when environmental factors become more favorable. Spores are resistant to radiation, drying, and disinfectants. Thermal resistance to denaturation is due to the high content of **calcium** and **dipicolinic acid** in the core. Spore formation is observed in **Bacillus** and **Clostridium species.**

B. **Initiation of sporulation** is related to the guanosine triphosphate pool. Regulation is by means of negative feedback. In addition, the availability of carbon and nitrogen sources are important to sporulation activity.

C. **Germination and outgrowth** occurs when environmental and nutritional factors allow for renewed cell growth.

1. Vegetative growth is triggered by the exposure to stimulants such as glucose, nucleic acids, and amino acids.
2. Activation of autolysin results in autolysis of the cortex. The synthesis of protein and structural components follows. The spore core membrane develops into the cell wall.

GENETIC TRANSFER

Genetic transfer refers to three principal mechanisms that result in the movement of genetic material into a host organism.

A. **Transformation** is the uptake and integration of naked DNA from the environment.

1. Once inside the cell, homologous recombination with the chromosome of the recipient must occur for the transformation to be successful.
2. Transformation can be induced in the laboratory with salt and heat shock. This technique is used to make cells take up plasmids carrying genes of interest.
3. There are natural transformers among both the Gram-positive and Gram-negative bacteria. The medically important natural transformers are: *Streptococcus* species, *Haemophilus* species, *Neisseria gonorrhea,* and *Helicobacter pylori.*

B. **Transduction** is the **phage-mediated** transfer of bacterial DNA. There are two kinds of transduction: generalized transduction and specialized transduction.

NOTE

The gene for diphtheria toxin is carried on a lysogenic bacteriophage.

IN A NUTSHELL

- ***Transformation*** → *Uptake and integration of naked DNA fragments*
- ***Transduction*** → *Phage-mediated exchange of information*
- ***Conjugation*** → *Direct bacteria to bacteria transfer of DNA (bacterial sex)*

1. In **generalized transduction,** bacterial DNA is mistakenly packaged into an empty phage head. This is a very low frequency event, but any gene can be transferred. Once inside the recipient cell, homologous recombination must occur for the transduction of information to be successful.
2. During **specialized transduction,** a lysogenic bacteriophage that is integrated into the bacterial chromosome excises itself, accidentally taking some chromosomal DNA. When the phage replicates, any bacterial gene that it has picked up is also replicated. These genes will be carried into cells that the progeny viruses infect. Specialized transduction occurs at a higher frequency that generalized transduction.

C. **Conjugation** is the **direct transfer** of bacterial DNA between organisms. It requires **cell-to-cell contact.** It is the most important mechanism for widespread transfer of genetic information between bacteria.

1. Most conjugation is **plasmid-mediated.** A plasmid is an extrachromosomal piece of circular DNA that can replicate itself. It often carries genes such as those that encode resistance to antibiotics, and virulence factors such as enterotoxins or adhesins. Plasmids vary in size, copy number per cell, and host range.
2. If a plasmid can exist only within a single species, it is called a **narrow-host-range plasmid.** If it can transfer between different genera of organisms, it is called a **broad-host-range plasmid.**
3. All plasmids can replicate themselves within the appropriate host, but not all plasmids can transfer themselves.
   a. A **conjugative plasmid** codes for the genes involved in transfer between cells.
   b. A **nonconjugative,** or mobilizable, plasmid requires the help of a conjugative plasmid to transmit itself to another bacteria.

D. **Insertion sequences** are small (1,000 bp) pieces of DNA that code for the enzyme **transposase,** which allows them to jump into and out of DNA.

1. A **transposon** consists of two insertion sequences flanking an antibiotic resistance gene.
2. Transposons and insertion sequences can insert into target DNA without significant homology at the site of insertion.
3. This ability to move between chromosomes, plasmids, or bacteriophages is an efficient mechanism for moving genes through a bacterial population. In fact, transposons are fre-

quently associated with the formation of multiple-drug resistance plasmids.

DENTAL CLINIC MICROBIOLOGY

A. Sterilization and disinfection

Dental instruments are cleaned and processed to remove pathogenic microorganisms. By far, the most common processing technique is **sterilization**, in which all life forms (bacteria, fungi, viruses and bacterial spores) are killed. By contrast, **disinfection** refers to killing of pathogenic organisms and most microorganisms in general. Disinfection will not kill bacterial spores, and disinfected instruments are not sterile. Instruments are often classified as follows:

1. Critical instruments—pierce mucus membrane or enter sterile tissues. Examples include scalpel blades, scalers, endodontic files.
2. Semicritical instruments—touch mucus membranes but generally do not enter sterile areas. Examples include dental mirrors and explorers.

Critical instruments must be sterilized or disposable. Semicritical instruments are usually sterilized but can be subjected to high level disinfection.

B. Sterilization methods

1. Autoclave (Steam)

   Autoclave uses steam under pressure. Typical temperatures and times are 121° for 20-30 minutes depending on load types.

2. Dry heat (Driclave)

   Dry heat requires both greater temperature and more time. Typical temperatures and times are 160° for 1-2 hours depending on load type.

3. Ethylene oxide (Chemiclave)

   Ethylene oxide requires times of 8-12 hours.

4. Miscellaneous

   "Cold sterilization" using long-term disinfectants is a misnomer. Spores are not killed by long disinfectant soaking, unless extremely long time periods are used. (Example: glutaraldehyde 12-15 hours).

All instruments must be cleaned before sterilization. Proteinaceous material, in particular, blocks heat from reaching microorganisms (example: blood, tissue). Other factors interfering with sterilization are overpacked autoclaves and use of an incorrect cycle (an exposure time which is too short).

C. **Disinfectant/Antiseptic/Sterilization**

Disinfectants, antiseptics and heat kill by a variety of actions including:

Material, method	Action
Steam Heat/most disinfectants	Protein denaturation
Ethylene oxide	Alkylation of proteins
Dry Heat	Protein denaturation/dessication
Glutaraldehyde	Alkylation/protein precipitation
Detergent	Membrane disruption
Soap	Emulsification of fat/ debris removal
Chlorhexidine	Membrane disruption
Mercury compounds	Protein precipitation

NOTE

Disinfectants are used on materials and surfaces while antiseptics have a similar function but are used on live tissue.

D. **Disinfectant guidelines**

1. Must be EPA registered
2. Should kill "benchmark" organism *Mycobacterium tuberculosis*
3. Should have ADA seal of approval for use on dental instruments.

E. **Sterilization monitors**

Sterilizers need to be checked to insure that they are adequately sterilizing instruments. There are two types of monitors:

1. Process Indicators—show that sufficient temperature was reached in that load. Often a color change strip or sections of autoclave bag. Does **not** show sterilization, only presence of high temperature for period of time.
2. Biological Monitors—Spore strips of spore-forming *Bacillus sp* are loaded with instrument load. Spores are cultured following autoclaving cycle. Negative culture is expected. Usually a "test strip" is autoclaved while a "control strip" is not (to show viability of spores).

Current requirement in most jurisdictions is **weekly** biological monitoring.

F. **Universal precautions**

Developed in the dental field following dental-related hepatitis B clusters in the 1970s, "universal precautions" is both a philosophy and a set of procedures. All patients are assumed to be potentially infectious and equivalent, sufficient disinfection/sterilization/cleaning procedures, etc. are completed for all patients regardless of the perceived or recorded health status of the patient. Preparation of rooms, instruments and materials depends on **procedure**, not **patient**.

1. Procedures included in universal precautions:
   a. Sterilization of most instruments.
   b. Disinfection of some instruments and "touch and splash" surfaces
   c. Barrier methods (gloves, masks, face shields, plastic chair covers, light handle covers)
   d. Disposable instruments (trays, prophy angles, etc.)

Bacteriology: Gram-Positive Cocci

There are two medically important genera of Gram-positive cocci—Staphylococci and Streptococci. Both are nonmotile and do not form spores. Staphylococci are catalase positive while Streptococci are catalase negative.

STAPHYLOCOCCUS

A. **Genus characteristics and classification**

1. Staphylococci are Gram-positive cocci that divide perpendicular to the last plane of division, forming clumps or clusters. Depending on the age of the culture, they can be observed singly, in pairs, in short chains, or in grape-like clusters.
2. They are hardy organisms because they are relatively resistant to heat and drying.
3. Metabolically, the Staphylococci are **facultative organisms,** and possess both **superoxide dismutase** and **catalase.**
4. Clinically, the most important distinction is between *S. aureus* and all other species that are nonpathogenic members of the normal flora. The **coagulase test** is a simple way to differentiate *S. aureus* from the coagulase-negative Staphylococci.
5. While there are six species of coagulase-negative Staphylococci, the most numerous species on the skin is *Staphylococcus epidermidis.* The other coagulase-negative species of clinical relevance is *S. saprophyticus.*

B. ***Staphylococcus aureus*** is a common infectious agent of humans, and tends to cause localized or toxin-mediated disease.

1. *Staphylococcus aureus* should not be considered normal flora, but it does transiently colonize the nasopharynx, skin, and vagina of up to 30% of the population.

2. Table 3-1 summarizes the conditions commonly caused by *S. aureus.*

Direct infection
Skin: Folliculitis, furuncles, carbuncles, abscesses, cellulitis, wound infection
Deep infection: Osteomyelitis (often post-trauma and/or surgery)
Systemic infections secondary to above
Osteomyelitis, endocarditis, lung abscesses, pneumonia
Toxin-mediated disease
Food poisoning, scalded skin syndrome, bullous impetigo, toxic shock syndrome

Table 3-1. Common conditions caused by Staphylococcus aureus.

3. *Staphylococcus aureus* does not produce a single factor that is necessary for virulence. The best host defense against infections are PMNs. No protective immune response is raised, so one can get infections again and again. Multiple virulence factors include: Protein A, binding protein, coagulase, DNAse, Staphylokinase, Hyaluronidase, Lipase, and various exotoxins (including hemolysins). *S. aureus* is generally resistant to penicillin and other β-lactase antibiotics.
4. **Treatment.** The drug of choice is a **penicillinase-resistant penicillin** (e.g., methicillin, nafcillin, or oxacillin) or a first generation cephalosporin. Methicillin-resistant *S. aureus* (MRSA) require therapy with vancomycin.

C. ***Staphylococcus epidermidis*** is most commonly a nosocomial pathogen.
1. The major virulence factor it produces is a viscous exopolysaccharide biofilm (slime).
2. When foreign bodies like IVs, catheters, and prosthetic valves are inserted into the host, *Staphylococcus epidermidis* can grow on their surface, embedded in the biofilm. This biofilm makes it difficult for the immune system to destroy the organism.
3. Treatment is with **vancomycin.**

D. ***Staphylococcus saprophyticus***
1. Causes **urinary tract infections** in sexually active women.
2. Treatment is with penicillin.

STREPTOCOCCUS

A. **Genus characteristics**

1. Streptococci are Gram-positive cocci that form **chains.**
2. Metabolically, the Streptococci are **aerotolerant anaerobes** because they derive energy from fermentation only (lack cytochromes).
   a. The principal end product of fermentation is lactic acid, and perhaps because of this they are more acid-tolerant than most bacteria.
   b. Although Streptococci can live in conditions where oxygen is present, they **lack catalase.** Catalase is a cytochrome-containing enzyme that degrades hydrogen peroxide to oxygen and water. This feature is useful for differentiating the Streptococci from other Gram-positive cocci such as the Staphylococci.
   c. Almost all medically important Streptococci are **auxotrophs,** meaning they require one or more vitamins, amino acids, or nucleic acids for growth, and therefore are not free-living in the environment.

B. **Classification.** The most common classification scheme for the Streptococci is based upon their reaction in blood agar.

1. **α-Hemolysis** – the red blood cells surrounding the colonies are intact, but there is partial breakdown of the heme, resulting in a green (viridans) pigment.
2. **β-Hemolysis** – the red blood cells surrounding the colonies are completely lysed. Beta-hemolytic Streptococci are also classified serologically into Lancefield groups (A-O) based on their cell wall carbohydrate. Clinically, the most important group is Group A.
3. **γ-Hemolysis** – no hemolysis or color change of the red blood cells is detected.

C. **α-Hemolytic Streptococci** can be distinguished from each other by their inhibition or growth in the presence of **optochin or bile.** This group includes *Streptococcus pneumoniae* and the Viridans Streptococci.

1. ***Streptococcus pneumoniae,*** also known as **pneumococci,** grow in pairs or short chains.
   a. **Transmission.** *Streptococcus pneumoniae* is spread person-to-person through **aerosol droplets.** 20-40% of normals are transiently colonized in their nasopharynx.

b. **Clinical manifestations.** It is the most common cause of **bacterial pneumonia,** and also causes **otitis media, sinusitis, bronchitis,** and **bacteremia.** It is the most common cause of **meningitis** in the elderly.

c. **Risk factors** for infection due to *Streptococcus pneumoniae* include poverty, a debilitated state of health, the absence of a spleen, and certain diseases such as sickle cell anemia, Hodgkin's disease, multiple myeloma, and AIDS.

d. The most important **virulence factor** of *Streptococcus pneumoniae* is its **carbohydrate capsule.**

e. **Prevention.** A **vaccine** of 23 of the polysaccharide antigens exists. It should be given to the elderly, those undergoing splenectomy, and those with a condition predisposing them to *Streptococcus pneumoniae* disease.

f. **Treatment.** In the past, all *Streptococcus pneumoniae* were sensitive to **penicillin.** Penicillin resistance due to transformation with DNA from nonpathogenic Streptococci in the oral pharynx is becoming a problem. These cases can be treated with vancomycin or erythromycin.

2. **Viridans Streptococci** are normal oral flora.

a. They produce dextran, a substance that allows them to adhere to many surfaces.

b. They are the major cause of **subacute endocarditis** (e.g., ***S. sanguis***) in those with abnormal heart valves. ***Streptococcus mutans*** causes **dental caries.**

c. Treatment of choice is penicillin.

D. **β-Hemolytic Streptococci** are further subdivided into groups A through D and F and G based on antibodies to a heat-stable, acid-stable carbohydrate in their cell walls. This antigen is called C carbohydrate or the Lancefield antigen.

1. **Group A Streptococci (GAS)** contains only one species, ***Streptococcus pyogenes,*** but it is the most important Streptococcal pathogen.

a. It can be distinguished from the other β-hemolytic Streptococci because it is **inhibited by the antibiotic bacitracin.**

b. **Clinical manifestations** of *S. pyogenes* are characterized as supprative and nonsupprative.

(1) **Suppurative** complications of **pharyngitis** include otitis media, peritonsillar cellulitis, peritonsillar and retropharyngeal abscesses, and bacteremic metastatic spread. Other supprative infections include **erysipelas** (skin infection), **pyoderma (impetigo), scarlet fever,** cellulitis,

IN A NUTSHELL

S. pneumoniae:
- *Alpha hemolytic; inhibited by optochin and bile*
- *Virulence conferred by polysaccharide capsule*
- *Clinical correlations:*
  - *Pneumonia (especially middle-aged and older adults)*
  - *Otitis media*
  - *Sinusitis*
  - *Meningitis (#1 cause in elderly)*
  - *Bacteremia*
- *Certain populations are especially susceptible: elderly, smokers, alcoholics, children, asplenics*
- *Vaccine available*
- *Treatment with penicillin but resistance on the rise*

NOTE

*Dextrans are involved in the ability of dental plaque (especially **S. mutans**) to adhere to enamel.*

lymphangitis, perianal cellulitis, puerperal sepsis, meningitis, pneumonia, and empyema.

(2) **Nonsuppurative** sequelae occur weeks after initial infection. Inflammation occurs in organs not originally infected. These sequelae include **acute glomerulonephritis,** in which edema, hypertension, and hematuria occur after pharyngeal or skin infection. Also classified within this group is **rheumatic fever,** occurring 7-28 days after pharyngitis and results in fever, carditis, and polyarthritis.

c. **Transmission and epidemiology.** Group A Strep is an obligate human parasite spread person-to-person by respiratory secretion via droplets, direct contact with the skin, or fomites. Pharyngitis is most common in winter and spring, with the highest incidence among preadolescents. Contaminated milk or eggs have also been the cause of food-borne epidemics of pharyngitis. Impetigo-like skin infections are most prevalent in summer and are often due to the infection of insect bites.

d. **Virulence factors.** The most important virulence factor to remember is **M protein.**

2. **Group B Streptococcus *(Streptococcus agalactiae)*** is part of normal vaginal and intestinal flora in 25% of a given population.

a. In contrast to *S. pyogenes, S. agalactiae* are **resistant to bacitracin.**

b. The major virulence factor is an **antiphagocytic polysaccharide capsule.**

c. **Infants** are more susceptible to disease from the organism than adults. They aspirate the organism during **passage through the birth canal,** and if they lack passive resistance from maternal IgG antibody, disease may ensue.

d. **Clinical manifestations** include **pneumonia, sepsis,** and **meningitis.** Group B Streptococci and *E. coli* are the major causes of these diseases in the neonatal population (under 1 month of age).

e. **Therapy** for *S. agalactiae* is a penicillinase-resistant synthetic penicillin.

3. **Enterococcus,** formerly Group D Streptococcus, include the important pathogens ***Enterococcus faecalis*** and ***Enterococcus faecium.***

a. Both organisms are part of the **normal fecal flora.** These organisms were classified as Group D, β-hemolytic Streptococci; however, hemolysis is not a consistent feature since it is encoded on a plasmid.

IN A NUTSHELL

S. pyogenes (Group A):

- *Beta hemolytic; sensitive to bacitracin*
- *Many virulence factors, including M protein, capsule, F protein, hyaluronidase, Streptolysins O and S, and erythrogenic toxins*
- *Clinical correlations*
  - *Pharyngitis (strep throat)*
  - *Scarlet fever*
  - *Skin: erysipelas, cellulitis, impetigo, necrotizing fasciitis, pyoderma*
  - *Secondary diseases: rheumatic fever and acute glomerulonephritis*
- *Treatment with penicillin*

IN A NUTSHELL

S. agalactia (Group B):
- *β-hemolytic; resistant to bacitracin*
- *Antiphagocytic capsule*
- *Causes meningitis, sepsis, and pneumonia in neonates (acquired during passage through vaginal canal where organisms are part of normal flora)*

Enterococci:
- *Variable hemolysis*
- *Normal fecal flora*
- *Cause urinary tract infections in hospitalized patients; rare cause of subacute endocarditis*

b. These organism can cause infection when they spread to the **urinary tract.** When the intestine is disrupted, they are one of the organisms found in an abcess. Enterococcus species also cause approximately 10% of cases of **subacute endocarditis.**

c. Unlike the Streptococci, Enterococci exhibit penicillin tolerance since they are inhibited, but not killed, by the antibiotic. Recently, vancomycin-resistant Enterococci have appeared.

Bacteriology: Gram-Positive Bacilli

The four most important genera of Gram-positive bacilli are: Listeria, Corneybacterium, Bacillus, and Clostridium. Listeria and Corynebacterium do not form spores, while Bacillus and Clostridium do.

LISTERIA MONOCYTOGENES

A. **Characteristics**

1. *L-monocytogenes* is a small Gram-positive coccobacillus that does not form spores.
2. Microscopically, the organism resembles nonpathogenic members of the Corynebacterium genus ("diphtheroids"), which are part of normal skin flora.
3. Unlike the diphtheroids, *Listeria monocytogenes* is **motile** at room temperature and produces β-hemolysis on blood agar.

B. **Transmission.** Listeria is a facultative **intracellular** pathogen; it infects phagocytic cells. It also produces *listeriolysin O*, which is a beta-hemolysin similar to streptolysin O. Listeria is most often acquired through ingestion of **contaminated meat or unpasteurized dairy products.**

C. **Risk factors.** Groups at risk of serious disease from *Listeria monocytogenes* include neonates, pregnant women, immunosuppressed patients, and alcoholics.

D. **Clinical manifestions**

1. **Neonatal infections** can arise by genital tract colonaization in pregnant women with transmission to the offspring across the placenta or during delivery.
   a. **Granulomatis infantiseptica** is an *in utero* infection presenting within two days of birth.

b. **Meningitis** in neonates will occur as a result of infection during delivery. It accounts for 10% of neonatal meningitides.

2. **Adult infections**
   a. **Meningitis** in immunocompromised hosts (steroids, chemotherapy, transplants, alcoholics, etc.). It also causes a small percentage of meningitis in neonates.
   b. **Bacteremia** in pregnant women

E. **Therapy** is ampicillin with sulfamethoxazole/trimethoprim as an alternative.

CORNEYBACTERIUM DIPHTHERIAE

A. **Characteristics.** *C. diphtheria* is a nonmotile, club-shaped, non-spore-forming, Gram-positive rod.

B. **Diphtheria toxin** is responsible for the clinical symptoms.

1. A **lysogenic phage encodes diphtheria toxin.**

C. **Clinical manifestations** of diphtheria include upper respiratory infection resulting in a **tonsillar grayish pseudomembrane** that may spread to the pharynx andlarynx, and can compromise the airway. Although the organism is not invasive, the toxin is absorbed systemically and acts on other tissues. Diphtheria toxin is especially toxic to the heart and can cause cardiac failure.

D. **Treatment** consists of **antitoxin** and erythromycin and should be administered as soon as possible.

E. **Prevention.** A vaccine containing diphtheria toxoid is administered during the first year of life. Boosters are required every ten years.

BACILLUS

Bacillus species are a group of large Gram-positive rods that produce **spores.**

A. ***Bacillus anthracis*** is the major pathogenic species.

1. Spores produced by the organism persist in soil or in products from infected herbivores for many years.
2. Encoded on a plasmid are an **antiphagocytic capsule** and three virulence factors that act in concert. What is commonly called anthrax toxin is actually a combination of these three toxins.
   a. **Protective antigen (PA)**
   b. **Lethal factor**
   c. **Edema factor**

NOTE

The antiphagocytic capsule of B. anthracis is unique in that it is composed of D-glutamate, not polysaccharide.

3. **Transmission.** Infection commonly occurs through skin cuts or abrasions, although the organism may also be inhaled.
4. **Clinical manifestations** of anthrax may be cutaneous or systemic.
   a. **Cutaneous anthrax** accounts for 95% of all infections. The characteristic presentation is papules that develop into ulcers with necrotic centers. Regional lymphadenopathy can occur. **Edema** is a major complication and this infection may be fatal in 20% of untreated cases.
   b. **Systemic anthrax** is acquired through the respiratory (inhalation anthrax-Woolsorter's disease) or gastrointestinal route, and results in lymphadenopathy and septicemia. It is almost always fatal.
5. **Therapy and prevention**
   a. The drug of choice for *Bacillus anthracis* infection is **penicillin.**
   b. Anthrax is controlled through prevention and clean up of contaminated areas.
   c. In the United States, a killed vaccine is available for individuals with a high risk of exposure and for livestock.

B. ***Bacillus cereus*** produces two enterotoxins and usually grows in foods, especially in cereal grains such as rice.
1. The principal **clinical manifestation** is **food poisoning** of two types:
   a. A short incubation food poisoning (1-6 hours; emetic type) causes severe nausea and vomiting.
   b. Food poisoning of long incubation (10-24 hours; diarrheal type) is observed as abdominal cramps and diarrhea.
2. **Therapy and prevention** include support for food poisoning, such as as administration of fluids.

CLOSTRIDIA

Clostridia are characterized as large, **obligate anaerobic, spore-forming** rods.

A. **Characteristics**
1. Clostridia are usually found in the soil or human GI tract.
2. The four major pathogenic species, *C. perfringens, C. difficile, C. tetani,* and *C. botulinum,* produce many toxins and destructive enzymes, including collagenase, protease, hyaluronidase, and lecithinase.

3. Most infections are mixed, that is, an aerobic organism grows first and reduces the environment, thus allowing the anaerobic Clostridia to grow.

B. ***Clostridium perfringens*** is a fast growing, nonmotile organism found in the soil and the intestine.

1. **Characteristics**
   a. There are five types, A through E, based on the production of **four toxins,** alpha, beta, epsilon, and iota. All strains produce **alpha toxin,** a calcium-dependent phospholipase C that is also known as **lecithinase.** It causes the lysis of erythrocytes and other cells.
2. **Transmission** of *C. perfringens* occurs through infection of disrupted skin, bowel, or other epithelial tissues. Infection may be due to traumatic injury, surgery, or septic abortion. The organism is part of the normal intestinal flora. Spores are frequently found in soil.
3. **Clinical manifestations** take on several forms depending on the site and severity of infection. A diffusely spreading organism may cause cellulitis and fasciitis and/or bacteremia.
   a. **Gas gangrene** or myonecrosis is a life-threatening illness characterized by muscle and connective tissue necrosis. Gas, an end product of fermentation, forms in the muscle tissue and causes **crepitation.** Approximately 80% of the cases of gas gangrene are caused by *C. perfringens.*
   b. **Food poisoning** can be caused by *C. perfringens.* It is thought to be the third most common cause of bacterial food-borne epidemics, after *Staphylococcus aureus* and Salmonella. The infection is characterized by abdominal pain and diarrhea for 12-24 hours; systemic effects are uncommon.
   c. **Skin and soft tissue infections** are typically localized infections. Tissue infection is characterized by crepitant cellulitis, stump infection in amputees, perirectal abscesses, diabetic foot ulcers, and decubitus ulcers.
   d. **Suppurative infection** is usually polymicrobial in origin. Intra-abdominal infection may cause bowel perforation and emphysematous cholecystitis. Pelvic infection may be seen as tubo-ovarian and pelvic abscesses and as septic abortion.
4. **Therapy** for gas gangrene consists of **surgical debridement plus pencillin.** Food poisoning due to the enterotoxin of type A is usually self-limiting, and hence is not treated with antibiotics.

C. ***Clostridium difficile***

1. *Clostridium difficile* is a component of the normal bowel flora in a small percentage of adults.
2. The organism can produce **two heat-labile toxins, enterotoxin (exotoxin A)** and **cytotoxin (exotoxin B).**
3. **Clinical manifestations.** *C. difficile* causes over 25% of **antibiotic-associated diarrhea** and 95% of the cases of **pseudomembranous colitis.**
   a. Two antibiotics that commonly precipitate pseudomembranous colitis are **clindamycin** and **ampicillin.**
   b. **Pseudomembranous colitis** presents with a history of nausea, vomiting, abdominal pain, and voluminous green diarrhea.
   c. Proctoscopy demonstrates pseudomembranes with an erythematous mucosa.
   d. Diagnosis is confirmed by demonstrating toxin in the stool.
4. **Treatment.** Oral **vancomycin or metronidazole** are the current treatments.

D. ***Clostridium tetani***

1. **Characteristics**
   a. Spores are abundant in the soil. When they are inoculated into a site of injury, a drop in the redox potential leads to germination. In 50% of cases, there is no history of a wound.
   b. The organism produces **tetanospasmin,** a plasmid-encoded **neurotoxin** that blocks the normal inhibition of spinal motor neurons. It prevents the release of the inhibitory neurotransmitters glycine and gamma-amino butyric acid, leading to **spastic paralysis.** Death can occur from respiratory failure.
2. **Clinical manifestations.** Tetanus has four clinical presentations:
   a. **Local infection** can cause persistant local muscle contraction. It has low mortality and is noninvasive.
   b. **Cephalic infection** is rare in presentation. It can follow chronic otitis media and can progress to the generalized form.
   c. **Generalized tetanus infection** is the most recognized form. It is painful and presents with **"lockjaw"** progressing to opisthotonos. Mortality is approximately 60%.
   d. **Neonatal tetanus** infects the umbilical stump. It is the major cause of infant mortality in developing countries.

IN A NUTSHELL

Virulence factors of Gram⁺ bacilli:

Corneybacterium diphtheriae →	*Phage-encoded toxin that inactivates EF-2 by ADP ribosylation. Protein synthesis inhibited in host.*
Bacillus anthracis →	*Antiphagocytic capsule*
Anthrax toxin (+)	*Protective antigen; Lethal factor; Edema factor (adenylate cyclase)*
Bacillus cereus →	*2 enterotoxins*
Clostridium perfringens →	*4 toxins; key one is alpha toxin (lecithinase)*
Clostridium difficile →	*Enterotoxin (exotoxin A) Cytotoxin (exotoxin B)*
Clostridium tetani →	*Plasma-encoded neurotoxin (tetanospasmin) blocks release of inhibitory neurotransmitters—spastic paralysis*
Clostridium botulinum →	*Neurotoxin blocks release of acetylcholine—flaccid paralysis*

3. **Therapy** for tetanus includes surgical debridement of the wound, the use of human **tetanus antitoxin, respiratory support** (if required), and **muscle relaxants** (curare-like drugs). The antibiotic treatment of choice is **metronidazole.**
4. **Prevention.** Since the same toxin is found in all strains, tetanus can be prevented by **immunization** with tetanus toxoid. Booster shots are required every ten years. In contrast to diphtheria, a patient who survives tetanus is not immune to the disease, since the minute amount of toxin necessary to cause disease is not sufficient to elicit an immune response.

E. ***Clostridium botulinum*** is found ubiquitously in soil.

1. **Characteristics**
   a. It produces a powerful, **heat-labile neurotoxin** that is usually ingested pre-formed with **improperly canned food.** The toxin blocks the release of acetylcholine from motor neurons in the peripheral nervous system, producing **flaccid paralysis.**
   b. There are seven immunologic types of toxins, some of which are phage-encoded.
2. **Clinical manifestations**
   a. There are a constellation of clinical signs associated with *C. botulinum* intoxication.
      (1) Dilated unreactive pupils (bulbar paralysis)
      (2) Descending weakness or paralysis usually starting with the cranial nerves
      (3) Progressive respiratory weakness
      (4) Absence of fever
      (5) Dry mucous membranes
      (6) Unexplained postural hypotension
   b. **Infant botulism** is also called **floppy baby syndrome.** When an infant is colonized by the organism, extremely low levels of toxin can be produced, leading to failure to thrive and eventually to progressive muscular weakness and poor motor development. Older children and adults do not seem to be afflicted. Infant botulism is the most common form in the U.S. and is the reason why infants should not be fed honey.
   c. In contrast to tetanus, the autonomic dysfunction that results from botulism is not life-threatening if the patient is intubated and appropriate ventilator management is used.
3. **Therapy** for botulism toxin poisoning includes respiratory support and human antitoxin therapy.

Bacteriology: Gram-Negative Organisms

The Gram-negative bacteria are a very heterogeneous group with a large number of medically relevant species. This chapter reviews the important Gram-negative cocci (Neisseria) as well as the various groups of Gram-negative bacilli (enteric organisms, zoonotic organisms, and respiratory organisms).

GRAM-NEGATIVE COCCI

A. **Neisseria**

1. **Genus characteristics**
   a. Neisseria are nonmotile, nonspore-forming, Gram-negative cocci. They are characteristically arranged in pairs (**diplococci**) with flattened adjacent sides facing each other. On Gram stain, they resemble coffee beans.
   b. Eight species have been identified. Two of these, ***N. meningitidis*** and ***N. gonorrhoeae,*** are pathogenic for humans.
2. **Isolation of the pathogenic Neisseria.** The pathogenic Neisseria are fastidious organisms. They are very susceptible to heat, cold, and drying, so specimens require special handling.

B. ***N. meningitidis*** (meningococcus)

1. **Features**
   a. The key virulence factor for *N. meningitis* is its **antiphagocytic capsule.** The capsule is the basis of serotyping and serves as the antigen for vaccines.
   b. Endotoxin and IgA protease are also important virulence factors.
2. **Transmission** of meningococcus is via **respiratory droplets.** The carriage rate in adult nasopharyngeal tissue is approximately 10-30%. Most carriers are asymptomatic. It enters the upper respiratory tract and may eventually disseminate. The risk of

disseminated disease is greatest in individuals with **late complement (C6, C7, and C8) deficiencies.**

3. **Clinical manifestations**
   a. **Meningitis,** usually of sudden and fulminant onset
   b. **Meningococcemia,** a vasculitic purpura causing disseminated intravascular coagulation (DIC)
   c. Meningococcus is responsible for **Waterhouse-Friderichsen syndrome,** characterized by coagulopathy, hypotension, adrenal cortical necrosis, and sepsis, which is usually fatal.
4. **Diagnosis** is made by identifying Gram-negative cocci in the spinal fluid and confirmed by culture of the CSF or blood and demonstration of oxidase production and maltose fermentation.
5. **Therapy and prevention**
   a. **Penicillin G** is the antibiotic of choice.
   b. Carriers and close contacts can be treated prophylactically with **rifampin.**
   c. A quadrivalent vaccine exists to capsule types A, C, Y, and W135. The vaccine does not protect against type B, which has a capsule that is not immunogenic.

C. ***N. gonorrhoeae*** (gonococcus)

1. **Features**
   a. Many strains produce β-lactamase
2. **Transmission** of the gonococcus is via **venereal contact,** but may also involve fomites. It has an increased incidence of infection in sexually active young adults (15-30 years old), nonwhites, low socio-economic groups, and urban settings.
3. **Clinical manifestations** are primarily associated with the urogenital tract, although disseminated disease can occur.
   a. **Acute urethritis and gonorrhea in men** is observed as a yellow **purulent discharge with dysuria.** Ninety percent of men are symptomatic. Common complications of acute urethritis include urethral stricture, epididymitis, and prostatitis. Proctitis is especially common in male homosexuals.
   b. **Gonorrhea in females** is asymptomatic in 20-80% of cases, most likely due to the inability to observe a discharge. Complications include pelvic inflammatory disease (15-20% of cases), generalized peritonitis, and infertility.
   c. **Disseminated disease** can be observed as meningitis, subacute bacterial endocarditis, and **arthritis.**
   d. **Ophthalmia neonatorum** results from maternal transmission to the infant during birth. Ophthalmic tetracycline, erythromycin, or silver nitrate is given to the neonate fo

prevention. Neonatal infection may cause gonococcal arthritis.

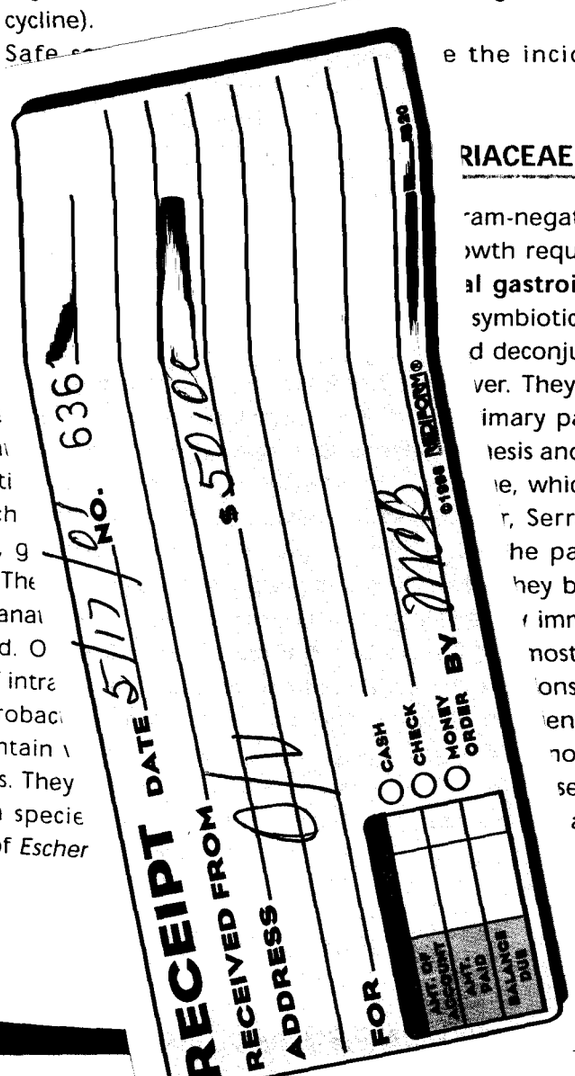
e. **Pharyngitis.** Diagnosis is established by identifying Gram-negative cocci in PMNs from the infected site and is confirmed by culture. This may occur in neonates, but is much more common in individuals who engage in oral sex.

4. **Diagnosis** of gonococcal urethritis in males is made by the observation of Gram-negative diplococci and neutrophils in the urethral exudate. In females, culture and biochemical testing is required due to the presence of other Gram-negative coccal forms as part of normal vaginal flora.

5. **Treatment and prevention**
   a. **Ceftriaxone** is the antibiotic of choice for treating infection. *N. gonorrhoeae* is no longer susceptible to penicillin because of two mechanisms: a plasmid-mediated β-lactamase, and chromosomally mediated decreased affinity of penicillin-binding proteins.
   b. Because combined infections are so common, treatment regime should include an antichlamydial agent (e.g., doxycycline).
   c. Safe s[illegible] e the incidence of

IN A NUTSHELL

	Gonococcus	***Meningococcus***
Spread	*Venereal; passage through birth canal*	*Aerosol*
Vaccine	*No*	*Yes*
Treatment	*Ceftriaxone*	*Penicillin*



GRAM[illegible] RIACEAE

The far[illegible] ram-negative, non-spore-f[illegible] owth requirements. The ma[illegible] **al gastrointestinal flora.** Ir[illegible] symbiotic relationship wit[illegible] d deconjugate bile salts an[illegible] ver. They also prevent the[illegible] imary pathogens. Coloniza[illegible] esis and receptor competiti[illegible] e, which include Escherich[illegible] r, Serratia, and Proteus, g[illegible] he pathogenic species. The[illegible] hey breach the normal anat[illegible] immunocompromised. O[illegible] nost common cause of intra[illegible] ons. A subset of Enterobac[illegible] ens because they contain [illegible] normal host defences. They[illegible] set includes Shigella specie[illegible] and some strains of *Escher*[illegible]

PLAN

A. **General characteristics.** There is no universally-accepted taxonomic classification for this group.

B. **Physiology**

1. The Enterobacteriaceae are facultative organisms; they can ferment or respire, depending upon the conditions.
2. Enterobacteriaceae are easily destroyed by heat and by common disinfectants or germicides. They are sensitive to drying or desiccation. In contrast, they survive best in a high-moisture environment. Respiratory care or anesthesia equipment are common sources of nosocomial infections. Contaminated ice machines or water supplies may harbor these organisms, causing epidemics.

C. **Antigenic structure**

1. **K antigens** (Vi antigen) are acidic **polysaccharide capsules.** They are antiphagocytic by blocking the access of complement or antibodies to the organism.
2. **H antigens** are the flagellar proteins and are present only in motile organisms. H antigen may exist in one of two phases in Salmonella.
3. **O antigen,** or somatic antigen, is the name for repeating oligosaccharide units found in the endotoxin in the outer membrane of Enterobacteriaceae.

D. **Pathogenicity** is due primarily to **endotoxin** (also called LPS; the lipopolysaccharide portion of the cell wall) present in all Enterobacteriaceae.

1. Toxicity lies in the lipid A portion. LPS may cause **endotoxic shock** when the enteric bacilli enter the bloodstream (septic shock). Endotoxic shock is characterized by blood pools forming in the microcirculation, resulting in hypotension. Vital organs lack adequate blood supply, leading to decreased tissue perfusion, acidosis, ischemia, and cellular hypoxia. DIC may also occur to further compromise the patient.
2. **Enterotoxins** are produced by some members of the family (some *E. coli,* Shigella) that exert their toxic effects on the small intestine. Enterotoxins cause secretion of fluid into the lumen, resulting in secretory diarrhea.
3. **Pili,** or fimbriae, promote adherence to tissues.

E. **Shigella**

1. **Characteristics**
   a. Shigella is an **obligate** human pathogen. There are four species of Shigella: *S. dysenteriae, S. flexneri, S. sonnei, and S. boydii.*

IN A NUTSHELL

All Enterobacteriaceae:
- *Gram-negative bacilli*
- *Facultative anaerobes*
- *Have endotoxin*
- *Found in GI tract (exception: Y. pestis)*

Some Enterobacteriaceae:
- *Motile (all except Klebsiella and Shigella)*
- *Have polysaccharide capsule (Klebsiella, Salmonella)—K antigen*

b. It is a slender, **nonmotile** organism.

2. **Transmission.** The primary mode of transmission is **fecal-oral.** These organisms are not killed by stomach acid, therefore, only a small number of organisms (< 100) are needed to cause disease.

3. **Pathogenesis.** The site of disease is in the colon, where Shigella invades and superficially destroys the intestinal mucosa. Virulence factors of Shigella include **adhesins, invasins,** and **toxins.** Most are encoded on plasmids. Finally, like all Gram-negative bacteria, Shigella have **endotoxin.** This endotoxin increases the local inflammatory response, but seldom causes septic shock since the organisms do not invade beyond the submucosa.

4. **Clinical manifestations** (shigellosis)
   a. **Bacillary dysentery** characterized by abdominal cramps and diarrhea. The feces contain blood, **polymorphonuclear leukocytes,** and mucus.
   b. One to four weeks after the disease, a **carrier state** may be set up if the organism is not cleared. Carriers experience long-term, recurrent bouts of disease.

5. **Therapy and prevention** includes hydration and electrolyte replacement.
   a. Ampicillin, tetracycline, or trimethoprim-sulfamethoxazole decreases the duration of symptoms and development of the carrier state.
   b. Prevention is facilitated through personal hygiene, proper garbage disposal, and water purification.

F. ***E. coli*** are motile enterobacteria. The GI tract is a natural reservoir for *E. coli.*

1. **Pathogenicity** in strains causing neonatal meningitis or uropathogenic strains is related to the presence of the **K1 capsular antigen,** which inhibits phagocytosis. Nephropathogenicity is associated with plasmid-mediated **hemolysin** production.

2. **Enterotoxigenic *Escherichia coli*** (ETEC) is a major cause of infant death in developing countries and is the most common cause of "traveler's diarrhea."
   a. More than 100 serotypes of *E. coli* cause this noninflammatory, secretory diarrhea that is similar to cholera, but less severe.
   b. The organism is acquired through the ingestion of fecally-contaminated food or water.
   c. Disease is noninvasive and occurs in the small intestine.

NOTE

Shigella and Salmonella typhi are the only Enterobacteriaceae that are human-only pathogens.

CLINICAL CORRELATE

An examination of a fecal sample for PMNs is a key component of the workup of infectious diarrhea. If they are present, you are dealing with an invasive organism such as Shigella, Salmonella, or Campylobacter. If there are no PMNs, you are more likely to be dealing with a toxin-producer such as E. coli or V. cholerae.

NOTE

An enterotoxin is a protein exotoxin that is released locally in the intestine.

NOTE

LT is a classic A-B toxin. The B portion binds to the epithelium in the small intestine. The A portion is enzymatically active and ADP ribosylates the adenylate cyclase regulatory protein, thus increasing cAMP levels in the cell.

IN A NUTSHELL

E. coli:

- *Most common cause of UTIs*
- *Most common cause of traveler's diarrhea*
- *Causes neonatal pneumonia, sepsis, and meningitis*

d. Major virulence factors are plasmid-encoded, including enterotoxins and pili-termed colonization factor antigens (CFA) that act as adhesins.

3. **Enteroaggregative *Escherichia coli*** (EAggEC) causes persistent **watery diarrhea in children and traveler's diarrhea.**
   a. The organism is acquired by ingestion.
   b. Virulence factors include pili, an enterotoxin, and a cytotoxin.
4. **Enteropathogenic *Escherichia coli*** causes **non-inflammatory diarrhea.**
   a. **Infants,** especially in the developing world, are very susceptible.
5. **Enterohemorrhagic *Escherichia coli*** (EHEC) causes **bloody diarrhea,** similar to dysentery.
6. **Enteroinvasive *Escherichia coli* (EIEC)** causes dysentery that is clinically indistinguishable from dysentery due to Shigella.
7. **Other clinical manifestations of *E. coli* infection**
   a. *E. coli* is the most common causative organism in **urinary tract infections (UTIs).**
   b. **Neonatal pneumonia** is usally the result of nosocomial infection due to aspiration during the birth process. Sepsis may also occur.
   c. **Neonatal meningitis** is also caused by exposure during birth. It is a serious infection resulting in 40-80% mortality.
8. **Therapy and prevention.** Antibiotic therapy is based on the site, severity, and sensitivity of the infectious organism.
   a. UTI is usually treated with Bactrim (sulfa + TMP) or a fluoroquinolone.
   b. Pneumonia, meningitis, and sepsis are commonly treated with a third-generation cephalosporin such as cefotaxime and/or an aminoglycoside.
   c. Diarrheal syndrome is usually treated with fluids and electrolytes only. Bismuth subsalicylate inactivates and binds enterotoxins.

G. **Salmonella.** Salmonella are **motile.**

1. **Nontyphoidal Salmonella** infections cause **inflammatory diarrhea** with fever and variable septicemia.
   a. Infection is acquired through the ingestion of eggs, chicken, and other contaminated food or water.
   b. In contrast to Shigella, a large inoculum (> 1 million cells) is needed to survive gastric acid and cause disease.
   c. Disease is more severe in children under the age of ten.

d. Known virulence factors include antiphagocytic capsules, outer membrane protein adhesins, and LPS. LPS and outer membrane proteins prevent complement-mediated killing during bacteremia. Also, the organism can grow within macrophages.

2. **Typhoidal Salmonella** infections are caused by **Salmonella typhi** and **Salmonella paratyphi** A, B, and C1. **Typhoid** or **enteric fever** is a progressive, subacute, febrile-wasting illness. It is common in developing countries, and the most severe manifestations occur in young children.
   a. **Transmission** is by ingestion of a large inoculum of bacteria in food or water contaminated by feces from a human.
   b. The intestine shows local inflammation with mucosal ulcerations and perforations. The organisms spread to the bloodstream and the reticuloendothelial system.
   c. LPS and other outer membrane proteins inhibit complement-mediated killing. **Vi antigen,** the polysaccharide capsule, inhibits phagocytosis and may contribute to the resistance of *S. typhi* to complement-mediated killing.
   d. Clinically, there are three phases of typhoid fever.
      (1) The **first week** is expressed as fever, lethargy, constipation, and pain.
      (2) The **second week** is when **bacteremia** occurs. Biliary and organ involvement arise along with a sustained fever with temperature/pulse dissociation (i.e., high fever, low pulse rate), abdominal pain, rose spots, and diarrhea. This period of time is when the GI tract is reinfected.
      (3) With the **third week,** exhaustion and fever improve unless other complications arise. Complications of typhoid fever include relapse (20%), severe bleeding, thrombophlebitis, abscess formation, pneumonia, cholecystitis (in acute and chronic colonization), and death (2-10%).
   e. **Treatment.** While chloramphenicol is still a first-choice drug for typhoid fever, the emergence of drug resistance has caused a shift to third-generation cephalosporins (e.g., ceftriaxone) and quinolones (e.g., ciprofloxacin) in developed countries.

H. **Common opportunistic Enterobacteriaceae**

1. **Genus Klebsiella** are nonmotile, lactose-fermenting rods. The colonies appear large and mucoid due to the presence of the **large capsule.** The major pathogen, ***Klebsiella pneumoniae,*** causes **severe lobar pneumonia** in individuals with underlying

NOTE

Unlike the other Salmonellae, S. typhi inhabit only the human colon.

NOTE

Salmonella causes osteomyelitis in sickle cell patients.

conditions such as alcoholism, diabetes, and chronic obstructive pulmonary disease.

2. **Genus Proteus** are highly **motile** organisms that cause **urinary tract infections.** Proteus species **produce urease,** which raises the urinary pH to levels that promote the production of **struvite.** These **stones** obstruct urinary flow and serve as a hiding place for the organism. Up to 10% of urinary tract infections are due to this organism.
3. Other genera of Enterobacteriaceae that are normal flora, but that can cause opportunistic infections, include Citrobacter (pyelonephritis), Enterobacter (pneumonia), and Serratia (pneumonia and UTIs).

MNEMONIC

The A's of Klebsiella:

- *Alcoholics*
- *Aspiration pneumonia*
- *Abscesses in the lungs*

Klebsiella pneumonia is sometimes characterized by thick, bloody sputum classically described as "currant jelly" sputum.

GRAM-NEGATIVE BACILLI: ADDITIONAL ENTERIC ORGANISMS

A. **Vibrio** have a highly characteristic comma-shape morphology. They are naturally found in both fresh and salt water and in several cold-blooded animals.

1. ***Vibrio cholerae*** are the most clinically important members of this group.
   a. **Pathogenicity** is related to the presence of a pilus that mediates adherence to the small intestine epithelium. It is a **noninvasive** infection with clinical effects mediated by the **enterotoxin** (choleragen).
   b. **Transmission** is via fecal-oral spread from contaminated water and food.
   c. **Clinical manifestations** of **cholera** are severe, **watery ("rice water") diarrhea** (20 liters/day) with the loss of sodium, chloride, potassium, and bicarbonate.
   d. **Therapy and prevention** includes rapid rehydration and electrolyte replacement. The antibiotic of choice is either **tetracycline** or **doxycycline.**
2. ***Vibrio parahemolyticus*** resembles *V. cholerae* structurally as a comma-shaped organism.
   a. **Clinical manifestations** are primarily diarrhea, attributed to ingestion of **raw or improperly handled seafood (especially shellfish).** The incubation period is 12-24 hours. Symptoms include explosive watery diarrhea (may be bloody), headache, abdominal cramps, fever, and vomiting.
   b. **Therapy and prevention.** Mild disease is usually self-limiting, subsiding within 2-4 days, with no therapy required. Organisms are usually sensitive to chloramphenicol, tetracy-

NOTE

Aeromonas and Plesiomonas are two other genera belonging to the Vibrionaceae family. Aeromonas hydrophilia live in water and can cause gastroenteritis and wound infections. Plesiomonas shigelloides is also associated with water sources and causes gastroenteritis.

cline, and cephalosporins. Adequate refrigeration of raw and cooked seafood aids in prevention.

B. **Campylobacter and Helicobacter.** Campylobacter are small, curved Gram-negative rods. The natural reservoirs are many domestic animals.

1. ***C. jejuni*** is spread person-to-person via the fecal-oral route.
   a. It is a primary pathogen that causes **enterocolitis,** an invasive enteritis with bloody diarrhea, crampy abdominal pains, malaise, and fever.
   b. Inflammatory proctitis is a clinical manifestation in homosexuals.
   c. Reactive arthritis may follow in individuals who have HLA-B27.
   d. It is gut flora of many domestic animals, including chickens, pigs, sheep, goats, and cattle.
   e. The antibiotic treatment of choice is either erythromycin or ciprofloxacin.
2. ***C. fetus*** is an opportunistic pathogen that causes bacteremia and metastatic infections in **immunocompromised patients.**
3. ***Helicobacter pylori*** is a spiral-shaped, motile rod that **produces urease**.
   a. Its reservoir in nature is unknown, and is possibly humans only.
   b. *H. pylori* exhibits age-dependent colonization rates and familial clustering. For example, it is present in the gastric mucosa of fewer than 20% of people less than 30 years old but increases to greater than 50% of people over 60 years old.
   c. It lives in the gastric mucus in close proximity to the gastric epithelial cells.
   d. Colonization may be asymptomatic; however, there is a high association with idiopathic chronic and acute **antral gastritis** as well as **duodenal ulcer** (90%).
   e. Therapy consists of bismuth salts, metronidazole and tetracycline, or amoxicillin.

C. **Pseudomonas** is a genus of Gram-negative rods that are widespread in soil and water. They are also a minor component of the bowel flora. Humans are very resistant to infection with Pseudonomas species. More than one host defense must be breached for infection to occur. The major host defenses are intact body surfaces, normal bacterial flora, complement lysis, and killing by polymorphonuclear leukocytes.

1. ***Pseudomonas aeruginosa*** is the most important member of the genus. It is an important nosocomial infection in **immunocompromised** and **chronically ill patients.**
   a. It possesses many virulence factors, and no single factor is decisive for virulence. Virulence factors include:
      (1) Pili-adhesin
      (2) Slime layer capsule-antiphagocytic alginate capsule; seen mainly in cystic fibrosis patients
      (3) Endotoxin
      (4) Exotoxins A and S inhibit protein synthesis.
      (5) Many enzymes (e.g., gelatinase, collagenase, phospholipase C, elastase); elastase is an important virulence factor because it allows the organism to invade blood vessels.
      (6) R factors
   b. **Risk groups** incude immunocompromised patients and hospitalized patients with underlying disease. Groups at particular risk are radiation therapy patients, **burn patients,** patients with metastatic or metabolic disease, patients on prolonged immunosuppressive or antimicrobial therapy, patients with prior instrumentation or manipulation, and patients with **cystic fibrosis.**
   c. **Clinical manifestations**
      (1) Wound and burn infections
      (2) Ecthyma gangrenosum—skin lesion with vascular invasion leading to hemorrhage and necrosis.
      (3) Ear infections—otitis externa (swimmer's ear, mild); in diabetics—malignant otitis externa
      (4) Pulmonary infections—especially in cystic fibrosis patients and the immunocompromised
      (5) Corneal infections in contact wearers
      (6) Urinary tract infections
   d. **Treatment** is dependent on antibiotic sensitivity of the individual isolate.

RESPIRATORY PATHOGENS

A. **Haemophilus**

1. **Characteristics.** Haemophilus are small pleomorphic coccobacilli.
   a. Many species of Haemophilus are normal flora of the human pharynx.
   b. The major pathogen of the group is ***Haemophilus influenzae.***

c. **Serotyping** in *Haemophilus influenzae* is based on the **polysaccharide capsules.** Disease due to *Haemophilus influenzae* is caused chiefly by **serotype b**. This capsule type prevents phagocytosis of the organism.

2. **Clinical manifestations.** Diseases caused by *Haemophilus influenzae* type b include **meningitis, otitis media,** and **epiglottitis.** Primarily children under the age of 5 are affected.
   a. **Meningitis.** Until the development of a vaccine, *Haemophilus influenzae* type b was the most common cause of bacterial meningitis in the 3-month to 6-year age group.
   b. **Acute epiglottitis** occurs with rapid onset and can compromise the airway.
   c. Nontypable strains cause pneumonia and otitis media. ***Haemophilus ducreyi*** is the cause of the veneral disease **chancroid.** It is characterized by painful, nonindurated, ragged ulcers confined to the genitalia and perianal areas.
3. **Treatment.** Recommended treatment for *Haemophilus influenzae* includes cefotaxime or ceftriaxone for meningitis or epiglottitis, and amoxicillin plus clavulanate for nonlife-threatening illnesses. Resistance due to β-lactamases is encountered in greater than 30% of isolates.
4. **Prevention.** A vaccine now exists for *Haemophilus influenzae* type b.

B. **Bordetella**

1. **Characteristics.** Bordetella are very small, Gram-negative fastidious coccibacilli. They are strict aerobes.
   a. ***B. pertussis*** causes **whooping cough,** and *B. parapertussis* causes a relatively mild disease, parapertussis.
   b. **B. pertussis** contains many virulence factors.
      (1) Attachment to the host is mediated by pili, also called fimbriae or filamentous hemagglutinins.
      (2) Toxins include pertussis toxin, adenylate cyclase toxin, tracheal cytotoxin, and lipopolysaccharide.
   c. **Clinical manifestations. Whooping cough** is the clinical manifestation of *B. pertussis* infection. This disease is highly communicable by the respiratory route, and humans are the only known reservoir. Whooping cough develops in three stages following an incubation period of 7-10 days.
      (1) **Catarrhal** or prodromal state occurs as a mild upper respiratory infection.
      (2) **Paroxysmal cough,** followed by the characteristic whoop on inspiration, then develops.

NOTE

Pertussis toxin, cholera toxin, and the heat-labile toxin of E. coli (LT) all work by ADP-ribosylation of G protein and the consequent increase in cAMP. There is a difference, however: cholera toxin and E. coli heat-labile toxin act by activating the stimulatory unit of G protein (G_s); pertussis toxin acts by inactivating the inhibitory unit of G protein (G_i).

(3) **Convalescence** is characterized by a slow decline in the "whoop." This stage lasts for months.

d. **Treatment and prevention. Erythromycin** is the drug of choice for *Bordetella pertussis* infection. However, antibiotic treatment has no effect on the course of disease if therapy is begun after the catarrhal stage. The best protection is **immunization.** There are two vaccines for pertussis, a killed-cell vaccine and an acellular vaccine.

CLINICAL CORRELATE

We have now reviewed all three elements of the DPT vaccine:
- Diphtheria
- Pertussis
- Tetanus

C. **Legionella** is a genus of Gram-negative bacteria that has over 40 species. The most important is ***Legionella pneumophila,*** a facultative intracellular parasite that uses the complement receptor to infect macrophages and then multiplies within the phagosome. It causes a wide spectrum of disease in humans.

1. **Characteristics**
   a. *Legionella pneumophila* is widely distributed in **aquatic environments.**
   b. It is an aerobic organism that requires iron and cysteine for growth.
2. **Transmission** is by aerosols.
   a. The source for humans is often **air conditioning equipment,** respiratory therapy devices, humidifiers, shower heads, or whirlpools.
   b. It is not transmitted person-to-person.
   c. Risk factors include immunocompromised status, alcohol abuse, and cigarette smoking.
3. **Clinical manifestations.** Disease caused by *Legionella pneumophila* varies greatly in its intensity. Infection can be asymptomatic.
   a. **Pontiac fever** is a mild, febrile illness without pneumonia caused by *Legionella pneumophila.*
   b. It causes a mild, atypical pneumonia that may progress to Legionnaires' disease.
   c. **Legionnaires' disease** is a severe, often fatal, pneumonia.
4. **Treatment.** The drug of choice for *Legionella pneumophila* is **erythromycin.**

Bacteriology: Anaerobes

Obligate anaerobes are bacteria that require a reducing environment. They cannot survive in an environment that contains oxygen because they are unable to detoxify the superoxide anion.

GENERAL CHARACTERISTICS

A. **Morphology and physiology**

1. This group includes Gram-positive and Gram-negative cocci, bacilli, and coil-shaped spirochetes that are often pleomorphic.
2. What unites these diverse organisms is the inability to detoxify the superoxide ion. The most essential enzyme in this process is **superoxide dismutase.** It catalyzes the conversion of the superoxide ion to hydrogen peroxide. The hydrogen peroxide can be further processed to water and oxygen through the action of catalase or peroxidase. Almost all obligate anaerobes lack these enzymes and hence require a reducing environment.
3. The greatest natural defense against anaerobic infection is healthy tissue that has a high oxidation-reduction potential and hence will not support the growth of the anaerobes.
4. Obligate anaerobes are normal inhabitants of anaerobic niches in the oral cavity, the vagina, and the gut. They can cause opportunistic infections in tissues adjacent to their normal habitat when they are displaced due to tissue injury or vascular compromise.

B. **Pathology**

1. The primary pathology is frequently **purulent abscess** formation.
2. Culture of the abscess most often reveals a polymicrobial infection with multiple facultative and anaerobic species.

IN A NUTSHELL

Oxygen is toxic to obligate anaerobes because they lack superoxide dismutase and catalase/peroxidase.

C. **Treatment.** Therapy includes **surgical drainage** plus antibiotics. Penicillin G, clindamycin, metronidazole, and chloramphenicol are usually the most effective antimicrobials. Cefoxitin is also effective against most anaerobes.

ANAEROBIC GRAM-NEGATIVE BACILLI

A. **Bacteroides** are the **primary organisms of the colon,** accounting for 30% of fecal isolates. Bacteroides are the most frequent cause of anaerobic infections and *Bacteroides fragilis* is the most common clinical infectious isolate.

1. ***Bacteroides fragilis*** is a Gram-negative, nonspore-forming, nonmotile bacilli.
   a. It inhabits the intestinal and genital tracts.
   b. It has four major virulence factors.
      (1) Unlike other group members, *B. fragilis* contains a **polysaccharide capsule** that is antiphagocytic and chemotactic. Capsular antigen alone can produce an abscess.
      (2) Since it is a Gram-negative organism, *Bacteroides fragilis* contains **endotoxin** (although it is much less toxic than the endotoxin produced by *E. coli*).
      (3) It can produce a small amount of superoxide dismutase, allowing it to survive oxygen exposures for long periods of time.
      (4) *Bacteroides fragilis* contains **β-lactamases** that confer resistance to penicillin.
   c. **Clinical manifestations** include intra-abdominal infections, including abscesses and peritonitis. It is a primary cause of Gram-negative bacteremia.
   d. **Therapy** consists of metronidazole with clindamycin or chloramphenicol alternatives. Surgical debridement and drainage may be necessary to eradicate the organisms.

2. ***Prevotella melaninogenicus,*** formerly ***Bacteroides melaninogenicus,*** is a small coccobacilli that can be found primarily in the oral pharynx.
   a. On blood agar, it forms **black pigmented colonies.**
   b. The virulence factors of *Prevotella melaninogenicus* include an antiphagocytic capsule and collagenase.
   c. *Prevotella melaninogenicus* is an important agent in oral and pulmonary infections.

NOTE

Bacteroides are part of the normal gastrointestinal flora. They can cause disease when there is a break in the surface of the mucosa.

NOTE

Prevotella (Bacteroides) melaninogenicus is suspected to be a primary factor in adult chronic periodonititis.

B. **Fusobacterium** are pleomorphic, Gram-negative rods with tapered ends. They normally inhabit the mouth, gastrointestinal tract, and female genital tract.

1. Fusobacteria do not have a capsule, but do possess an extremely potent endotoxin.
2. ***Fusobacterium nucleatum*** is the most common isolate. It is an important agent in oral infections, lung abscess, and other pleuropulmonary infections.
3. ***Fusobacterium necrophorum*** is often found in liver abscesses. It contains both a leukocidin and a hemolysin.
4. Most isolates of Fusobacteria are susceptible to penicillins, cephalosporins, and clindamycin.

NOTE

Fusobacteria are often found in diseased periodontal pockets.

ANAEROBIC GRAM-POSITIVE BACILLI

A. **Spore-forming.** Clostridium species were discussed previously in the Gram-Positive Bacilli chapter.

B. **Nonspore-forming**

1. **Propionibacteria** normally inhabit the skin. These organisms may infect shunts and prosthetic devices, and are a cause of **acne.**
2. **Actinomyces**—see next chapter.

ANAEROBIC COCCI

A. **Anaerobic Gram-positive cocci**

1. **Peptostreptococcus** are the only genus of anaerobic cocci that are important in clinical infections.
   a. They are almost always isolated from a **mixed infection.**
   b. As a group, they are frequently recovered from cutaneous, oral, respiratory, and female genital tract infections.
   c. Peptostreptococci are sensitive to penicillin, cephalosporins, clindamycin, and metronidazole.

B. **Anaerobic Gram-negative cocci. Veillonella species** are small bacteria that resemble the Neisseria species. Part of normal mouth, nasopharynx, and vaginal flora, they are rarely a cause of infection. They may be confused with Neisseria on Gram stain.

Bacteriology: Mycobacteria and Actinomycetes

The mycobacteria are acid-fast bacilli notable for the high-lipid content of their cell wall. They are responsible for tuberculosis (*M. tuberculosis*), disseminated disease in AIDS patients (*M. avium-intracellulare*), and leprosy (*M. leprae*). Actinomycetes are Gram-positive organisms with a characteristic branching filament growth pattern that resembles fungi. These organisms are abundant in soil. The Actinomycetes include two genera of clinical relevance: Actinomyces and Nocardia.

MYCOBACTERIA

A. ***Mycobacterium tuberculosis***

1. **Characteristics**
   a. **Obligate aerobes** and **acid-fast bacilli (AFB)**. Once stained, *M. tuberculosis* resist decolorization with an acidic alcohol rinse (hence the name acid-fast, based on ability to retain the stain). This property is dependent on the **waxy lipid cell wall,** which includes **mycolic acids.**
   b. The cell wall contains lipoproteins or glycolipoproteins essential for tuberculin activity and confers the ability to induce **Type IV hypersensitivity reactions** (DTH, delayed-type hypersensitivity).
   c. *M. tuberculosis* is **slow-growing,** requiring 20-60 days before growth can be visualized; its doubling time is 18 hours.
2. **Antigenicity**
   a. **Purified-protein derivative (PPD)** of the cell wall is the preferred antigenic material for **skin testing.**

BRIDGE TO IMMUNOLOGY

Type IV hypersensitivity reactions are discussed in detail in the Basic Immunology chapter of the Microbiology/Immunology review book.

3. **Pathogenicity**
   a. **Cord factor** is associated with virulence and characteristic serpentine grouping pattern that is seen in virulent strains. Cord factor inhibits polymorphonuclear leukocytic migration, elicits granuloma formation, and attacks mitochondrial membranes.
   b. **Sulfatides** act synergistically with cord factor and protect the microorganism from attack by the hydrolytic enzymes of the lysosome.
4. **Epidemiology.** *M. tuberculosis* is found only in humans. It is more common in lower socioeconomic groups. There has been a rapid, recent rise in cases in the U.S., partially related to the AIDS epidemic and to immigration patterns.
5. **Transmission**
   a. Occurs primarily through **droplet nuclei** inhalation.
   b. The most infectious person is one with untreated cavitary pulmonary tuberculosis who is actively expelling bacilli.
   c. The risk of infection is much greater than the risk of disease (i.e., more people are infected than have clinical disease) since disease may be related to weakened immune responses.
6. **Pathogenesis** is dependent on immunologic responses. First, delayed-type hypersensitivity (a T-cell immune response) occurs within 3-4 weeks after infection and correlates with positive tuberculin reaction. Acquired cellular immunity is associated with "resistance" or protection from reinfection.
   a. **Primary infection**
      (1) **Exudative type** occurs when the organism is inhaled and spreads via alveolar macrophages, to the hilar lymph nodes. Hematogenous dissemination may occur at this time; however, signs of infection are minimal and immune competent hosts will successfully limit the organism to the pulmonary location.
      (2) **Productive type** is characterized by the tubercle forms (with or without caseation) and depends on the host's immune response.
      (3) The primary site of infection forms **calcified lesions** (referred to as **Ghon complex**). Delayed-type hypersensitivity develops and infection becomes quiescent in pulmonary as well as metastatic sites (the PPD test is now positive). Immunocompromised or debilitated patients may have progressive primary disease from local sites or more distant sites without the disease becoming quiescent.

CLINICAL CORRELATE

The primary lung infection is usually found subadjacent to the pleura in the lower part of an upper lobe or in the upper part of a lower lobe of one lung. This localization reflects the areas receiving the greatest flow of air.

b. **Secondary infection (reactivation)** is usually localized, particularly in **lung apices,** due to the higher oxygen tension (PO_2). Tubercle formation occurs histologically with **caseation, necrosis,** and **fibrosis.** Secondary infection results from either a breakdown of quiescent foci or from new infection, despite the acquisition of cellular (T-cell) immunity.

7. **Clinical features of tuberculosis.** The clinical presentation may include nonspecific constitutional symptoms such as fatigue, weight loss, anorexia, weakness, fever, and night sweats.
   a. **Pulmonary TB.** Eighty-five percent of cases are pulmonary, although infection may involve any organ of the body. Pulmonary disease may present with cough, hemoptysis, and pneumonitis.
   b. **Miliary or disseminated masses** develop anywhere, but some sites are favored, signifying an advanced stage of the disease. These favored sites include bone and joints (osteomyelitis), meninges (meningitis), kidneys, peritoneum, and lymph nodes.

8. **Diagnosis**
   a. **Abnormal chest x-ray**
   b. **Acid-fast bacteria (AFB)** in sputum; culture of *M. tuberculosis.*
   c. **Skin testing**
      (1) PPD-S, the designated standard (purified-protein derivative) is injected under the skin. Tuberculin tests should be read from 48-72 hours after intradermal injection.
      (2) The interpretation is based on **diameter of induration** and recorded in millimeters; 10 mm or greater in diameter of induration is considered positive for *M. tuberculosis* infection (but not necessarily active disease); 5-10 mm is considered as doubtful signficance.
      (3) **False negative reactions** are usually the result of tuberculin injections too deep into skin. Other causes include immunosuppression or anergy due to overwhelming infection, steroids, malignancy, etc.
      (4) **False positive reactions** are usually caused by hypersensitivity to mycobacteria other than *M. tuberculosis.* False positives can also result from previous immunization with **BCG (Bacille Calmette-Guerin) vaccine** (not administered in the United States, but widely used outside U.S.).
      (5) **Diagnostically, a positive PPD test does not prove active disease.** The tubercle bacilli must be isolated and identified in culture.

NOTE

Eighty percent of pulmonary TB cases in adults are due to reactivation of an infection acquired years or even decades earlier.

regimens. Currently, the drugs considered "first line" include:

- *Isoniazid*
- *Rifampin*
- *Ethambutol*
- *Pyrazinamide*

problem that can best be controlled by sensitivity testing of the isolate from the patient. When resistance is detected, additional drugs (ethionamide, streptomycin, ciprofloxicin) may be added to the regimen.

10. **Prevention**
    a. **INH prophylaxis** of household contacts of patients with newly diagnosed active disease or treatment of recently converted (within the past two years) PPD-positive individuals.
    b. **BCG immunization.** This treatment is given only to PPD-negative individuals in countries where incidence of tuberculosis is high. It is used to establish cell-mediated immunity to TB, although it must be remembered that individuals will become skin test-positive for PPD (thus eliminating its utility as a diagnostic tool).

B. ***Mycobacterium bovis***

1. ***M. bovis*** is the etiologic agent of **tuberculosis in cattle.** It can also cause human TB, usually through the ingestion of unpasteurized, contaminated milk.
2. The most common clinical manifestations are lesions in the cervical and mesenteric lymph nodes with possible dissemination to the bones and joints. Pulmonary TB is also possible through the inhalation of infected droplets (for example, by dairy farmers).
3. *M. bovis* will result in a positive PPD skin test.
4. The BCG vaccine is derived from a live, attenuated *M. bovis* strain.

C. ***Nontuberculous mycobacteria* ("atypical").** The nontuberculous mycobacteria encompass approximately 20 species, including *M. kansasii, M. marinum, M. scrofulaceum, M. fortuitum-chelonei* and most importantly, *M. avium-intracellulare*. They cause mycobacteriosis. The agents of mycobacteriosis are not spread from human to human; they are environmental agents.

1. **General characteristics of non-TB mycobacteria.** There is no known primary animal host. The organisms usually occur as natural inhabitants of the soil.

monly occurs in children.

c. **Cutaneous lesions** are caused by *M. marinum* when this organism contaminates an open wound; the disease is called "swimming pool" granuloma.

d. **Disseminated disease** can arise from *M. kansasii* or from *M. avium-intracellulare,* particularly in patients with acquired immunodeficiency syndrome (AIDS).

3. **Treatment of nontuberculous mycobacterium.** Many of the mycobacteria are resistant to the usual antituberculosis drugs. Antibiotic regimens may require as many as six drugs, including rifampin (which is quite effective against *M. kansasii*) and clarithromycin (good against *M. avium-intracellulare* complex). Surgical resection is also recommended on occasion.

D. ***Mycobacterium leprae***

1. **Species characteristics**
   a. Cannot grow *in vitro* on any culture medium.
   b. Organisms are acid fast and induce a delayed-type hypersensitivity in patients.

2. **Leprosy (Hansen's disease)**
   a. Leprosy is endemic in Africa, South and Southeast Asia, and South America. Small endemic areas are found in the U.S. (Hawaii, Texas, California, Louisiana, and Florida).
   b. **Transmission** is through contact with organisms from nasal secretions or ulcer exudates from infected individuals. Lesions classically involve the **cooler regions of the body,** including the skin of nasopharynx, cartilage, eyes, testicles, and larynx. Incubation period for the infection to progress to clinical disease is 5-7 years.
   c. **Disease forms**
      (1) **Tuberculoid leprosy** is indolent and nonprogressing. Clinical findings show mature **granuloma in the dermis,** which also contains epithelioid cells, giant cells, and lymphocytes (predominantly T_{DTH} cells).
      (2) **Lepromatous leprosy** is a progressive and invasive disease. Pathologic examination shows foamy histiocytes with an absence of epithelioid and giant cells. Cell-mediated immunity is suppressed (**negative lepromin skin test**) and T8 (suppressor cells) lymphocytes infiltrate

cells. The disease is marked by a low infectivity rate and occurs more frequently in individuals with defective cell-mediated immunity.

e. **Therapy** requires long-term (3-5 years) antibiotic treatment to eradicate the organism. Tuberculoid leprosy responds to **dapsone** plus **rifampin**. Lepromatous leprosy is treated with dapsone plus rifampin and **clofazimine**. Close contacts should also be treated.

ACTINOMYCETES

(Actinomyces, Nocardia, Streptomyces) are characterized by their filamentous form.

A. **Actinomyces.** Several species from this genus can cause actinomycosis. The most important species are ***A. Israelii*** *and A. naeslundii.*

1. **Species characteristics**
   a. *A. Israelii* are anaerobic, nonacid fast, Gram-positive bacilli.
   b. They are part of the **normal oral flora** (not found in soil) and are usually pathogenic only after oral trauma.
2. **Clinical manifestations (termed actinomycosis)**
   a. **Cervicofacial actinomycosis** causing lower jaw involvement following the development of dental caries (about 50% of actinomycotic infections) or after dental work. **Pyogenic abscesses** may develop with swelling, tenderness, and erythema. **Granules** consist of Gram-positive mycelial filaments surrounded by eosinophils and leukocytes. **Osteomyelitis** (bone infection) is a frequent occurrence. Chronic, poorly healed oral cavity abscess will spread by direct extension. Fortunately, the incidence of oral actinomycosis is declining due to improved oral hygiene practices.
   b. **Thoracic actinomycosis** is caused by the extension from cervicofacial infection in approximately 20% of cases.
   c. **Abdominal actinomycosis** is due to traumatic perforation of the intestinal mucosa, such as a ruptured appendix or perforated ulcer.
   d. **Pelvic actinomycosis** arises in women with intrauterine devices.

NOTE

Common extension of oral infections will occur to the skin, where characteristic "sulfur granules," can be seen.

B. **Nocardia.** ***N. asteroides*** is the most common isolate found in soil and aquatic environments.

1. **Characterisitics.** Nocardia are aerobic, Gram-positive, **partially acid-fast** filamentous organisms.
2. **Clinical manifestations** (nocardiosis)
   a. Half of patients have underlying disease. It is an opportunistic infection in patients with hematological malignancies. Seventy-five percent of cases occur in males.
   b. Infection begins as a **chronic lobar pneumonia** following inhalation of the organism and may be subclinical.
   c. The central nervous system is the most common site of metastatic infection, occurring hematologically. The kidneys and skin may also become involved (in metastatic infection). **Abscess formation** is the most common pathologic finding.
3. **Treatment** is usually with sulfonamides and may require surgical drainage of abscesses.

IN A NUTSHELL

- *Actinomyces are anaerobes; Nocardia are not.*
- *Nocardia are partially acid-fast; Actinomyces are not.*

Rickettsia and Chlamydia are obligate intracellular parasites. Rickettsial diseases include Rocky Mountain spotted fever, typhus, cat scratch disease, and Q fever. Chlamydia are responsible for ocular trachoma and are the number one cause of sexually transmitted diseases in the U.S.

RICKETTSIACEAE

A. **General characteristics and physiology**

1. Rickettsiaceae are a family of small, pleomorphic Gram-negative coccobacilli.
2. The Rickettsiaceae family comprises four genera: Rickettsia, Bartonella, Coxiella, and Ehrlichia. The members of the family share several unique characteristics.
   a. All members are **transmitted by arthropods,** except Bartonella (where direct contact is the mode of transmission); *Coxiella burnetii,* is acquired by humans primarily through aerosol inhalation, although it has an arthropod vector in its natural hosts.
   b. Most species are **obligate intracellular parasites** of endothelial cells (Rickettsia) or leukocytes (other genera).
   c. Most cause zoonotic disease with humans as an accidental host. **Chlamydia trachomatis** is the exception; it is a human pathogen with no reservoirs.
   d. **All are susceptible to tetracyclines** (e.g., doxycycline).

B. **Rickettsial diseases**

1. **Rocky Mountain spotted fever (RMSF),** is caused by ***R. rickettsii*** and accounts for 95% of rickettsial diseases in the U.S.
   a. **Epidemiology.** RMSF is found throughout the United States, particularly in the south central and eastern portions of the country.

with malaise, frontal headache, and fever.

(2) Skin manifestations appear within 2-4 days after onset of symptoms. Initially, a **maculopapular** rash appears on the hands and feet (including palms and soles) and **spreads centripetally** to involve the trunk.

(3) Other manifestations may include splenomegaly, hepatomegaly, thrombocytopenia, and disseminated intravascular coagulation.

2. **Epidemic typhus** (louse-borne typhus) is caused by ***Rickettsia prowazekii.***
   a. **Transmission.** The agent is transmitted by the **human body louse**. *Rickettsia prowazekii* are found within the louse feces and enter individuals via skin excoriations. The louse is the vector, but not a reservoir, for *Rickettsia prowazekii* (humans are the primary reservoir).
   b. **Clinical features** are similar to those of RMSF but the rash is less prominent and spares the palms and soles.
   c. **Brill-Zinsser disease** is the recurrent form of *R. prowazekii* infection. *R. prowazekii* can remain dormant in lymph nodes.
3. **Endemic** or **murine typhus** is caused by ***R. typhi*** cycled by the rat and its ectoparasites (through feces).
   a. **Transmission.** The **rat flea** is the most common vector. It becomes infected when it feeds on the reservoir—the rat—during the acute part of the rat's illness. Organisms are passed in the flea feces, and can be scratched in at the site of the bite.
   b. **Clinical manifestations** are similar to but less severe than in epidemic typhus.
4. **Scrub typhus** is caused by ***R. tsutsugamushi.*** It occurs endemically in Asia.
   a. **Transmission.** *R. tsutsugamushi* is transmitted by **mites** (chiggers). Since there is transovarial passage of the organism, the mite is a reservoir as well as the vector. Other reservoirs include rats, field mice, and shrews. Humans are accidental hosts.
   b. **Clinical features.** Clinically, the disease resembles epidemic typhus.

CLINICAL CORRELATE

Lesions in the palms of the hands and the soles of the feet are valuable clinical clues. Causes include RMSF, meningococcemia, and secondary syphilis.

ticks, small wild animals, sheep, cows, and goats.

b. **Clinical features**

(1) Q fever is characterized by vague symptoms such as fever and chills, headache, malaise, and myalgia. It is unique among the Rickettsial diseases in that it **does not cause a skin rash.**

(2) Many cases are asymptomatic or present with a self-limited febrile illness.

(3) The classic presentation of the disease is as a **pneumonia** with fever and no pulmonary symptoms. Atypical pneumonia and a rapidly progressive pneumonia also occur.

(4) Chronic Q fever can manifest as myocarditis or hepatitis.

6. **Human ehrlichiosis** is caused by ***Ehrlichia chaffeensis.*** It has a **tick vector.** The clinical course can range from asymptomatic to fatal. The classic presentation includes fever, headache, and myalgia. Important laboratory features are leukopenia, thrombocytopenia, and anemia. Fewer than half of patients develop a rash.

7. Bartonella henselae causes **cat scratch disease** (a benign local lymphadenopathy that follows contact with cats) and **bacillary angiomatosis.** The latter occurs in AIDS patients and is characterized by proliferative vascular lesions in the dermis (resembles Kaposi's sarcoma) and internal organs.

C. **Treatment.** Treatment with antibiotics have greatly reduced the morbidity and mortality due to rickettsial infections. While all rickettsial diseases were traditionally treated with tetracycline, newer antibiotic regimens include doxycycline, ciprofloxacin or chloramphenicol for Rickettsia and Ehrlichia. *Coxiella burnetii* can be treated with doxycycline or ciprofloxacin.

CHLAMYDIAE

A. General characteristics and physiology

1. Chlamydiae **are obligate intracellular parasites** infecting birds and mammals.

2. They possess a Gram-negative envelope that contains a genus-specific lipopolysaccharide but **lacks muramic acid.**

NOTE

Q fever is an outlier among the Rickettsiaceae:

- *It does not have an arthropod vector.*
- *It does not cause a rash.*

IN A NUTSHELL

Organism	Vector	Disease
R. rickettsii	*Tick*	*Rocky Mountain spotted fever (think rash – palms/soles moving to trunk)*
R. prowazekii	*Louse*	*Epidemic typhus (think rash – trunk spreading to extremities)*
R. typhi	*Flea*	*Endemic (murine) typhus*
R. tsutsugamushi	*Mite*	*Scrub typhus*
E. chaffeenis	*Tick*	*Ehrlichiosis*
C. burnetii	*None*	*Q fever (no rash!)*
B. henselae	*None*	*Cat scratch fever and bacillary angiomatosis*

infectious and reproductive forms. The infectious form is known as the **elementary body (EB).** It is incapable of multiplication. The intracellular form capable of binary fission is called the **reticulate body (RB).** This form is not infectious.

B. ***Chlamydia trachomatis.*** *Chlamydia trachomatis* is transmitted by fomites, **sexually, or perinatally.** It infects humans only. Infected cells develop oval **vacuolar inclusions** that contain glycogen and hence will stain with iodine.

1. **Clinical features**
   a. **Ocular trachoma** is the leading cause of **blindness in developing countries.** It is caused by repeated infections that result in chronic conjunctivitis, leading to scarring, eyelid deformities, and progressing to pannus formation and total blindness.
   b. **Inclusion conjunctivitis**
      (1) In infants, it is associated with maternal genital infection. If untreated, it may develop into pneumonia.
      (2) Adult conjunctivitis is often associated with contaminated swimming pools and hot tubs.
   c. **Sexually transmitted diseases.** *C. trachomatis* is the number one cause of STDs in the U.S.
      (1) In men: **nongonococcal urethritis,** epididymitis, prostatitis, and proctitis.
      (2) In women: cervicitis, urethritis, salpingitis, and pelvic inflammatory disease.
      (3) **Lymphogranuloma venereum** (LGV) is a venereal disease. LGV is more common in males and blacks. The primary lesion is a **painless, vesicular site** on any part of the genitalia, anus, or rectum. The lesion becomes painful and suppurative and can spread into the inguinal and femoral lymph nodes.
2. **Treatment and prevention.** *C. trachomatis* has been traditionally treated with erythromycin or tetracyclines. Its spread can be minimized through improved hygiene and safe sexual practices.

C. ***Chlamydia psittaci.*** *Chlamydia psittaci* is transmitted by inhalation of the organisms from **infected birds** and their droppings.

1. **Risk factors.** Anyone coming in contact with an infected bird is at risk, but those that have close contact with birds such

NOTE

Birds + pneumonia → think C. psittaci

rales.

b. **CNS manifestations** are characterized by severe frontal headaches. Toxic encephalitis may occur, leading to death.

D. ***Chlamydia pneumoniae.*** *Chlamydia pneumoniae* is a human-only pathogen that is believed to be transmitted by inhalation. This organism is a cause of pharyngitis, bronchitis, and a relatively mild atypical pneumonia, although life-threatening infections have occurred.

E. **Treatment.** The drugs of choice for Chlamydia infections are erythromycins or tetracyclines.

NOTE

Epidemiologic studies have also associated atherosclerosis and coronary artery disease with antibody to Chlamydia pneumoniae, and this organism has been found in plaques of coronary arteries. However, its role in the pathogenesis of atherosclerosis is still unknown.

The spirochetes are motile, helically coiled organisms that divide by transverse fission. Spirochetes contain an axial fibril, an outer sheath, a protoplasmic cylinder (cell wall and membrane), and cytoplasm. There are three genera of spirochetes that can cause disease in humans: Treponema (syphilis, yaws, pinta, bejel), Borrelia (Lyme disease, relapsing fever), and Leptospira (leptospirosis). This chapter focuses primarily on the two most clinically relevant spirochetes: *Treponema pallidum,* the etiologic agent of syphilis, and *Borrelia burgdorferi,* the tick-borne cause of Lyme disease.

TREPONEMA

A. ***Treponema pallidum*** is the most important species. It has a capsule-like outer coat and a tapered end. Organisms are highly motile and constantly rotate about an axial filament. An important characteristic is that *T. pallidum* **does not grow on artificial media** and therefore cannot be cultured in the laboratory. *T. pallidum* is the etiologic agent of *syphilis.*

1. **Transmission and epidemiology**
   a. Syphilis is primarily a sexually transmitted disease. It can also be transmitted across the placenta and rarely, from blood transfusions.
   b. Risk groups include individuals with multiple sex partners and infants born to infected mothers.
2. **Clinical manifestations**
   a. **Primary syphilis** arises within 2-10 weeks following exposure. Organisms spread locally from the site of inoculation to the lymph nodes and bloodstream. A **chancre** forms at the site of inoculation. Initially, the chancre is a firm, **painless** reddish lesion with a raised border. The center usually

CLINICAL CORRELATE

The rash of secondary syphilis appears on the palms of the hands and the soles of the feet (as in Rocky Mountain spotted fever and meningococcemia).

b. **Secondary syphilis** occurs 1-3 months following primary syphilis. Symptoms represent disseminated disease and commonly include **rash,** fever, sore throat, headache, and generalized lymphadenopathy (especially in the epitrochlear region). White patches occur on mucous membranes (condylomas). **Condyloma lata** occur in moist areas, including the anus, vagina, axilla, and mouth. These lesions are highly infectious and are teaming with treponema.

c. **Latent syphilis** develops in 30-40% of infected individuals. Mucocutaneous relapses are most common, with the lesions remaining infectious. After two years, contagious lesions rarely develop.

d. **Tertiary (late) syphilis** arises in approximately 30% of untreated cases of primary syphilis.

(1) **Benign tertiary syphilis** is marked by indolent **granulomatous lesions (gummas)** in the skin, mucocutaneous tissue, liver, or skeletal system. Spirochetes are rarely seen.

(2) **Cardiovascular syphilis** is manifested as aortitis and occurs 10-20 years after untreated primary disease. Medial necrosis can occur with the destruction of elastic tissue. Saccular or fusiform aneurysms may develop and rarely dissect.

(3) **Neurosyphilis.** Can occur more than 20 years after the initial infection. One or more CSF abnormalities may be apparent. Other neurologic symptoms are **"Argyll-Robertson pupils"** and **tabes dorsalis.** In the former condition, the pupil constricts on accommodation but fails to react to light. Tabes dorsalis is observed as a wide-based gait with long "slapping" motions of legs upon ambulation.

e. **Congenital syphilis** results from the transplacental transmission of spirochetes to the developing fetus. It most commonly occurs if the mother has primary or secondary disease. There is approximately 25% mortality if left untreated, with a high incidence of spontaneous abortions and stillbirths. Adequate treatment of the mother will prevent disease in the fetus. Early manifestations in the newborn include hepatosplenomegaly, hemolytic anemia, pneumo

nonspecific and antigen specific. While **darkfield microscopy** can be used to visualize organisms from lesions, these serologic tests are the most common method of diagnosis.

a. **Nontreponemal tests** use **cardiolipin** as the antigen and can be performed as a complement fixation (CF) test or as a flocculation test (called **VDRL** or **RPR assays**).

b. Fluorescent antibody tests (**fluorescent treponemal antibody, FTA**) are specific for treponemal antigens. The assay detects specific antibodies and is used for the confirmation of positive nontreponemal tests. Once positive, it remains positive for life and therefore cannot be used to monitor therapeutic response.

4. **Treatment and prevention**

a. **Penicillin** is still the drug of choice since all treponemes are highly sensitive organisms.

b. Spread can be minimized through use of safe sexual practices.

B. Other treponemal diseases

1. **Yaws** is caused by *T. pertenue* in the tropics and is transmitted by direct contact. It is mainly a disease of children and is manifested as a painless erythematous lesion usually on the arm or leg.
2. **Pinta** is caused by *T. carateum* and is acquired by person-to-person contact, rarely via sexual transmission.
3. **Bejel** is caused by *T. pallidum* subspecies endemicum. Poor hygiene plays a role and is responsible for endemic syphilis in the Middle East, Africa, and Southeast Asia. Transmission is by direct contact; skin lesions are highly infectious.

BORRELIA

A. General characteristics

1. Borrelia are transmitted by **arthropods** to humans.
2. Borrelia are coarse, irregular coils that are very flexible and motile.

b. The highest incidence of disease is found during the spring and summer when the nymph and adult stages of the tick require blood meals.
c. The disease was first described in Lyme, Connecticut and is now found throughout the U.S. and in Europe and Australia.
d. It is not uncommon for the patient to have no memory of a tick bite.

2. **Clinical manifestations**
   a. The hallmark initial finding is **erythema migrans** (a red macule, progressing to annular erythema with central clearing) at the site of the tick bite. The rash occurs within the first 10 days of infection and fades within 3-6 weeks. Infection, however, is still active. Constitutional symptoms include fever, headache, malaise, myalgias, adenopathy, and mild meningeal irritation. These early symptoms typically last for approximately 4 weeks.
   b. Untreated infection may lead to late neurologic and cardiac disease.
      (1) **Neurologic symptoms** include severe headaches, meningitis, cranial nerve palsies, and painful peripheral neuropathies. These symptoms resolve after several months.
      (2) **Cardiac symptoms** include fluctuating cardiac arrhythmias (resolving after several weeks), myocarditis, and pericarditis.
   c. Late-stage disease is marked by arthritis, occuring weeks to years after disease onset.
3. **Diagnosis** of Lyme disease is made from the clinical picture and a history of tick bite or exposure, in combination with laboratory identification by serology.
4. **Therapy** consists of doxycycline for early disease; also amoxicillin, cephalosporins, and erythromycin.

C. **Relapsing fever** is named for its numerous antigenic shifts and variations. It is caused by *B. recurrentis* and is transmitted by the **human body louse.**

1. **Clinical manifestations**
   a. The incubation period is approximately one week (3-1 days) and is marked by the acute onset of high fever, seve headache, myalgia, photophobia, cough, and meningism

d. Subsequent fevers are less intense and last a shorter period of time. Antigenic shifts occur during this time. The afebrile times allow a new virulent variant to emerge and cause disease.

2. **Therapy.** Treatment with tetracycline or erythromycin rapidly leads to recovery.

Mycoplasmataceae are the smallest free-living organisms. They are prokaryotic cells resembling Gram-negative bacteria, but they lack the cell wall. The family contains the genera **Mycoplasma** and **Ureaplasma.** The organisms are classed into one or the other genera based on the ability to hydrolyze urea. The species of medical importance in humans are *Mycoplasma pneumoniae, Mycoplasma hominis,* and *Ureaplasma urealyticum.* Disease in humans involves the respiratory and urogenital tracts.

MYCOPLASMATACEAE

A. **General characteristics and physiology**

1. Morphologically, they are filamentous and pleomorphic. They **lack cell walls** and are therefore penicillin resistant.
2. They are facultative organisms that are mainly fermentative.
3. These organisms are unique among the prokaryotes because they **require sterols** for growth and their cell membranes contain cholesterol.

B. ***Mycoplasma pneumoniae***

1. **Epidemiology and transmission.** *Mycoplasma pneumoniae* is found throughout the world. Transmission occurs via aerosol droplets. Clinical illness is more likely in ages 5-20.
2. **Pathogenesis.** Once in the upper respiratory tract, *Mycoplasma pneumoniae* are found among the cilia of the epithelial cells.
3. **Clinical manifestations.** *M. pneumoniae* is the most common cause of **pneumonia in young adults** (walking pneumonia).

monitis in a teenager is a strong indication of *M. pneumoniae* infection.

4. **Diagnosis** is primarily based on clinical findings and serology.
5. **Treatment** consists of macrolides (e.g., **erythromycin** or azithromycin), tetracyclines, or fluoroquinolones. Since the family Mycoplasmataceae lacks a cell wall, all members are resistant to the β-lactam antibiotics.

C. ***Mycoplasma hominis***

1. Sexually transmitted agent
2. A major source of infection in postpartum women.
3. Clinical manifestations include postabortal and postpartum fevers and bacteremia, as well as pelvic inflammatory disease.
4. Treatment with tetracyclines; in contrast to other Mycoplasma, it is resistant to macrolides.

D. ***Ureaplasma urealyticum***

1. Sexually transmitted agent
2. Unlike other Mycoplasma, it produces urea
3. A minor cause nongonococcal urethritis
4. Treated with tetracycline or erythromycin

IN A NUTSHELL

M. pneumoniae:

- *Lack cell walls*
- *Transmitted via respiratory droplets*
- *Cause atypical pneumonia—#1 cause of pneumonia in young adults*
- *Cold agglutinins (IgM) used in presumptive diagnosis*
- *Treatment: erythromycin, tetracycline, or fluoroquinolones*

Viruses are the smallest agents of infection, ranging from 20-300 nm in diameter and consisting of either RNA or DNA surrounded by a protective protein shell (capsid). The protein shell may be surrounded by an envelope containing lipid and protein. Virus multiplication occurs only within host cells, and is accomplished by the separate synthesis and subsequent assembly of component parts. Some viruses have the unique capacity to become latent and even to integrate their genomes into the host cells. In this situation, the integrated viral genome is replicated as part of the host genome and is transmitted to each daughter cell without production of infectious virus.

CLASSIFICATION AND IDENTIFICATION

Classification and identification of viruses is based on common characteristics shared by viral families (such as the presence of single- or double-stranded viral nucleic acids, DNA or RNA).

A. **Morphology** is based on structures and components (nucleic acids, envelope, etc.) common to the viruses and is a basis for viral classifications. See Figure 11-1.

1. **Terminology**
   a. **Virion** is a term used to describe the complete infectious virus particle.
   b. **Capsid** is the **protein shell** that encloses and protects the nucleic acid genome (either RNA or DNA). The individual protein units are called **capsomeres.** These structures protect the viral genome from destruction in the extracellular environment. Capsids also control the host range and cell

IN A NUTSHELL

Viruses:

- *Smallest infectious agents*
- *Only one type of nucleic acid (DNA or RNA)*
- *No metabolic activity outside of living cells; obligate intracellular parasite*

2. **Nucleocapsids** have characteristic symmetry that is usually **helical** or **icosahedral** (see Figure 11-1).

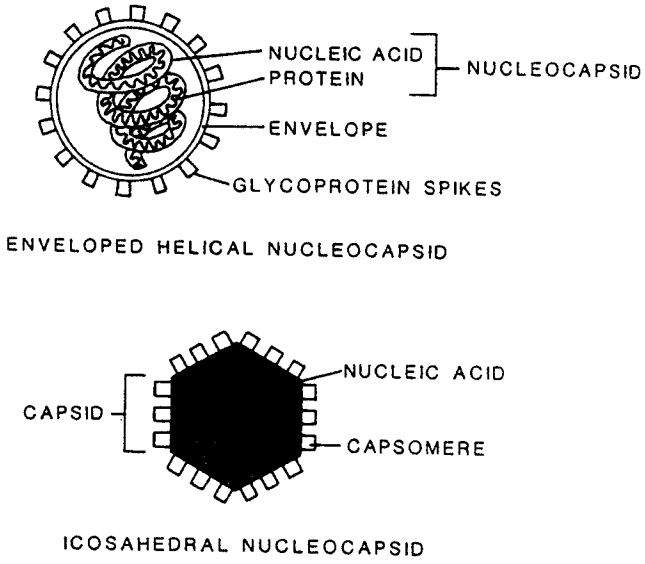


Figure 11-1. Viral nucleocapsids.

   a. A **helical nucleocapsid** is marked by an extended nucleic acid cavity surrounded by helically arranged proteins with an outer lipid envelope. Examples of viruses with helical structure include ortho- and paramyxoviruses and rhabdoviruses.
   b. **Icosahedral symmetry** is marked by condensed nucleic acids forming a central portion of cuboidal nucleocapsid structure. The icosahedral structure may be enveloped or naked. Examples of icosahedral symmetry include parvovirus, adenovirus, herpesvirus, and picornavirus.

3. **Envelopes** are lipid-containing structures surrounding some viral particles.
   a. Envelopes are derived from nuclear or plasma cell membranes acquired during viral maturation. The viral envelope is usually acquired when the viral nucleocapsid buds through the host's membrane.

below) and entry into host cells.

d. **Lipids** are the major component of the viral envelope. They are a complex mixture of phospholipids, glycolipids, and neutral lipids that are a part of the host cell membrane in which the virus multiplied.

4. **Virus classification** is based on nucleic acid composition such as the presence of single-stranded or double-stranded DNA or RNA. **Positive-sense RNA (+RNA)** serves directly as mRNA, while **negative-sense RNA (-RNA)** must use an RNA polymerase to synthesize a complementary positive strand to serve as mRNA.

5. **Viral proteins** are important in the initial contact with the host cell; they dictate which cells will be infected. Viral proteins also determine the antigenic structure of a virus and protect the genome against host nucleases.

   a. **Hemagglutinins** are viral proteins that agglutinate red blood cells. The hemagglutinin of influenza virus promotes attachment of the virus to host cells. It can also function in the fusion of the virion with the host cell membrane. They are excellent vaccine antigens.

   b. Enzymes serve several biological functions for the virus.

      (1) **Neuraminidase** acts to hydrolyze sialic acid. It functions to help release virus particles from the cells in which they were formed to further propagate infectivity.

      (2) **RNA polymerase** is required for viral replication of negative-sense RNA viruses. This enzyme must be brought into the cell as a part of the virion.

      (3) **Reverse transcriptase** in retroviruses transcribes single-stranded RNA into double-stranded DNA. Transcribed DNA can then be integrated into the host genome by an **integrase** enzyme.

B. Replication. Viruses are dependent on the host cell to provide the synthetic mechanisms and metabolic machinery for replication.

1. The **replication cycle** of the virus may either lyse the host cell or form a stable interaction that allows the host cell to survive. A stable interaction causes the viral genome to become incorporated into the host cell. The virus may be latent (e.g., her-

tein p24 is used to determine the virus load in the blood.

In a Nutshell

- *All RNA viruses have single-stranded RNA except for Reoviruses (ds)*
- *All RNA viruses are enveloped except Reoviruses, Caliciviruses, and Picornaviruses*
- *All DNA viruses have double-stranded DNA except Parvoviruses (ss); Hepadnavirus has ss regions in the DNA*
- *All DNA viruses have an icosahedral nucleocapsid except Poxviruses*
- *All viruses with helically symmetrical nucleocapsids are RNA viruses*

tissue-specific (e.g., poliovirus attaches primarily to CNS and GI tract cells).

b. **Penetration and uncoating** follows the adsorption step and is typically mediated by receptor-specific endocytosis. The virus usually loses its coat or envelope directly following penetration. Uncoating separates the capsid (and envelope) from the nucleic acids.

c. The **synthetic period** begins when uncoating is complete. Its time course is variable depending upon the virus. Specific mRNA must be transcribed from viral nucleic acid by host cell mechanisms. Macromolecular synthesis begins with an eventual accumulation of viral components (including empty capsids or nonenveloped nucleocapsids). Minus-strand RNA virus, double-stranded RNA viruses, and DNA viruses initiate nucleic acid synthesis to produce mRNA, while positive-sense RNA viruses can immediately initiate protein synthesis. This stage is marked by an orderly sequence of synthetic events (with some proteins synthesized early and others later in the synthetic processes).

d. **Production of viral proteins**

(1) In **positive-sense RNA viruses** like polio, the viral RNA (mRNA) is read directly by the host cell ribosome and enzymes for RNA synthesis are produced. Other proteins that are synthesized at this time will inhibit host biosynthetic processes, while still others will serve as capsid (structural) proteins.

(2) In other viral groups, the viral genome (–RNA, double-stranded RNA, or DNA) must first synthesize the messenger RNA molecules. They do this by an RNA-dependent RNA polymerase (transcriptase) that is contained in the virion and encoded by the viral genome in the case of RNA viruses; or by transcription of the viral DNA to synthesize the mRNAs necessary for protein synthesis. Some of the proteins will be structural units (capsomeres, peplomeres), others will be enzymes necessary for progeny DNA synthesis (DNA polymerase).

e. **Replication of viral genome (nucleic acid)**

(1) **Plus-stranded RNA viruses** can immediately begin protein synthesis without nucleic acid replication or tra

IN A NUTSHELL

*Positive-sense RNA viruses **are** mRNA and can directly encode all the proteins needed for replication. Other viruses require enzymes such as RNA-dependent or DNA-dependent RNA polymerases to produce mRNA for viral replication.*

(2) **Minus-strand and double-stranded RNA** viruses must first synthesize mRNA for the eventual translation into viral proteins. The minus-strand acts as a negative template for synthesis of mRNA. These viral genomes carry RNA-dependent RNA polymerase required to synthesize mRNA from the (–) strand.

(3) Double-stranded RNA viruses (REO and Rota) synthesize a positive strand of RNA from the negative strand of the parent. This acts both as mRNA and as the replicative intermediate to make the negative-sense RNA that will be assembled with a complimentary positive strand to make the progeny genome.

(4) Retroviruses use the negative strand of the DNA intermediate to make positive-sense progeny RNA.

(5) Double-stranded DNA viruses replicate by the same process employed by the host cell; each strand serves as the template for synthesis of the complimentary DNA copy. Hepatitis B virus contains a viral RNA-dependent DNA polymerase (a reverse transcriptase) that uses the viral mRNA as a template to synthesize the missing portion of the viral genome, which is then duplicated by host cell DNA polymerase.

(6) Single-stranded DNA viruses (Parvovirus) synthesize a double-stranded intermediate to use as the replicative template for the single-stranded DNA progeny.

f. **Viral assembly** occurs toward the end of the synthetic period. Complete intracellular virus assembly begins. The viral genomes and capsid polypeptides assemble, forming infectious viral offspring.

g. **Release** of the complete nucleocapsid is the final stage of the replication cycle.

(1) Enveloped viruses are released gradually by a budding process. Nucleocapsids bud through virally altered membrane patches, thus gaining viral specific glycoproteins.

(2) Poxviruses and naked capsid viruses burst out rapidly from the cell, causing the cell to disintegrate.

NOTE

- *All DNA viruses replicate entirely in the nucleus except for Poxviruses.*
- *All RNA viruses replicate entirely in the cytoplasm except for Influenza viruses and Retroviruses.*

IN A NUTSHELL

Viral growth cycle:

- *Attachment of virus to cell*
- *Penetration of cell*
- *Uncoating of viral genome*
- *Transcription of genome into mRNA*
- *Translation into proteins*
- *Replication of viral genome*
- *Assembly of particles into new viruses*
- *Release of virus*

DNA VIRUSES

A. Adenoviruses

1. **Characteristics.** Adenoviruses are medium-sized linear **double-stranded DNA** viruses with a **naked icosahedral nucleocapsid.** They contain fibers that serve as viral attachment sites. There are 41 adenovirus serotypes, with approximately one-third accounting for the bulk of human disease.

2. **Transmission** usually occurs person-to-person through **respiratory and ocular secretions** (usually infecting mucous membranes or lymphoid tissue). Humans are the only known host.

3. **Clinical manifestations**
   a. **Acute respiratory disease** that may induce a latent infection in tonsils, adenoids, and other lymphoid tissues. Most infections are acute and self-limited. Influenza-like illness occurs in the late fall and winter, characterized by pharyngitis, fever, cough, and malaise.
   b. Acute febrile pharyngitis may be seen in infants and children.
   c. Conjunctivitis ("pink eye")
   d. Diarrhea and gastroenteritis
   e. Pneumonia and pharyngoconjunctivitis are less frequent complications except in the military, where frequent epidemics may occur.

4. **Diagnosis** is made by clinical presentation, culture of stool, urine, throat swabs, or conjunctival scrapings. Serology can be performed by ELISA or complement fixation techniques.

5. **Treatment and prevention**
   a. Treatment is supportive.
   b. A vaccine consisting of live, nonattenuated viruses is used in the military. An asymptomatic intestinal infection results that induces respiratory tract immunity.

B. Papovavirus family includes Papilloma, Polyoma, and Vacuolating viruses.

1. **Characteristics.** Papovaviruses are small **double-stranded circular DNA** viruses with a **naked icosahedral nucleocapsid.**

2. **Human papillomaviruses** (HPV) are distributed worldwide. They cause skin warts (prevalent in children) and genital warts (condyloma acuminata) and are the most common cause of **viral** STD.
   a. **Transmission** occurs via contact with warts.

MNEMONIC

To remember which viruses are RNA vs. DNA, just memorize the DNA viruses (there are fewer) and know that all the rest are RNA viruses. To help remember the DNA viruses, think about how HHAPPP you will be once you finish studying. The tough part is recalling that the PPP represents papova-parvo-pox (picorna and paramyxo are RNA viruses).

b. In addition to warts, HPV has also been implicated in benign laryngeal papillomas.
c. A growing number of serotypes are associated with penile, laryngeal, and, especially, **cervical cancer.**
d. **Treatment** includes removal by electrocautery, cryotherapy, or chemicals. Recurrence is common.

3. **BK virus** is distributed widely in the human population, with 75% serologic positivity in children aged 6 and older. Infection remains latent. Disease (virus in urine) is limited to **patients undergoing immunosuppressive** therapy and is thought to be a reactivation of infection acquired in childhood.

4. **JC virus** is a polyoma virus associated with **human progressive multifocal leukoencephalopathy.**

C. **Herpesviruses**

1. **Characteristics**. Herpesviruses are large **double-stranded DNA** viruses with an **enveloped icosahedral nucleocapsid**. Herpesviruses are characterized by **latent infections** with recrudescence of disease (increasingly prevalent in immunocompromised patients).

2. **Herpes simplex virus, types 1 and 2 (HSV-1 and HSV-2)** cause **oral and genital lesions** by infecting epithelial cells. Upon the resolution of acute illness, latent infections are commonly found in **neurons**. Humans are the only known host. Direct contact with the infected lesion or secretions are necessary for transmission.
   a. **HSV-1** is typically acquired early in life. It is usually associated with **oral lesions ("fever blisters")** and with neurological disease. HSV-1 is the leading cause of **sporadic encephalitis** in the U.S. Note that HSV-1 can cause genital lesions as well.
   b. **HSV-2** is acquired after the onset of sexual activity. It is associated with **genital lesions** and with neurological disease. It is transmitted through sexual contact.
   c. **Primary infection** may be asymptomatic or characterized by **vesicular lesions** with edema, leading to ulceration and crusting. The lesions heal without scarring. Infection is characterized by **primary gingivostomatitis (HSV-1)** or by **primary herpes genitalis (HSV-2).** Neonatal herpes may be acquired *in utero* or during birth.
   d. **Recurrent infection** occurs at the site of primary infection. It involves the activation of latent virus from neurons of cervical or sacral ganglia. Stresses that lead to reactivation

NOTE

Dentists are often asked by parents to diagnose primary gingivostomatitis in young patients. Painful oral lesions and fever will be present in a young patient (usually < 4 years). The disease is self limiting.

include hormonal changes (menses), fever, sunlight, physical trauma, and immune suppression.

e. **Diagnosis** is made by identification of the clinical lesions as well as with viral isolation by tissue culture. The **Tzanck smear** will demonstrate **multinucleated giant cells** on the stains of scraped lesions (differential includes varicella-zoster virus). Immunofluorescent stain of the lesions will demonstrate viral **intranuclear inclusion bodies**. The latter test is preferred.

f. **Antiviral therapy** with **acyclovir** is instituted upon diagnosis in serious infections (see the Antimicrobials chapter).

3. **Varicella-zoster virus (VZV)** is isolated from patients with **chickenpox** and **shingles**. Shingles is reactivation of latent varicella infection.

a. **Chickenpox** (varicella) caused by herpes zoster virus is a mild self-limited illness in children expressed as fever followed by a **macular then papular eruption** on skin and mucous membranes. Chickenpox usually occurs in epidemics and is highly contagious. The virus is spread by **respiratory secretions** with approximately a 2-week incubation period. The papules are pruritic and become vesicular on skin and mucous membranes (all stages of the lesions are found simultaneously). Disease is more severe in adults, with **pneumonia common in immunocompromised patients**. Encephalitis is a rare but severe complication.

b. **Shingles** is a recurrent infection, usually in adults, that may be activated by trauma, neoplasm, drugs, or immunosuppression. Shingles occur from virus that remains latent in the sensory ganglia of spinal or cranial nerves. Severe **dermatomal pain** occurs with **vesicular eruption**, fever, and malaise. Pain and neuralgia may precede dermal eruption by 1-3 days. The pain may persist for months (postherpetic neuralgia), especially in older patients.

c. **Diagnosis** is primarily made by serologic assay and by the history of exposure to the virus. Scrapings of lesions stained with fluorescein-labeled antivaricella antibodies will reveal multinucleated giant cells with viral intranuclear inclusions.

d. **Treatment and prevention**

(1) Varicella infections can be treated with acyclovir.

(2) Immunosuppressed patients can be treated prophylactically with human varicella-zoster immunoglobulin (VZIG).

(3) An **attenuated varicella vaccine** was approved for use in the U.S. in 1995. Children should be vaccinated betwee

12 and 18 months of age because maternal antibody can interfere with attenuated viral vaccine efficacy.

4. **Epstein-Barr virus** is the etiologic agent of **infectious mononucleosis (IM).** IM is spread by saliva and respiratory secretions and is initiated in the oropharynx. EBV replicates in epithelial cells prior to infecting the B lymphocytes. EBV infection is associated with **Burkitt's lymphoma** and **nasopharyngeal carcinoma** in particular populations. It is also the etiologic agent of **hairy oral leukoplakia** in immunocompromised hosts.
   a. **Clinical manifestations** last 2-4 weeks and include fatigue, malaise, tender lymphadenopathy, pharyngitis, fever, headache, and splenomegaly. Hepatitis and meningitis are less frequent clinical symptoms. **Atypical lymphocytes** with a foamy cytoplasm (Downey cells) are noted on peripheral blood smear.
   b. **Nonspecific serologic responses** to EBV infection are due to the viral infection of B lymphocytes. Heterophile antibodies that arise will agglutinate sheep and horse red blood cells (as measured by the classic Paul-Bunnell test).
   c. **Specific antibodies** also arise to the viral capsid proteins. IgM class antibodies indicates recent infection, while IgG antibodies persist for more than 1 year.
   d. **Diagnosis.** Ten percent or more of patients fail to make heterophile antibodies. In these individuals diagnosis is made by ELISA assay, isolation of virus, and/or nucleic acid hybridization technology.
   e. **Treatment** is supportive. Acyclovir can be used in severely ill patients.

5. **Cytomegalovirus (CMV)**
   a. CMV infection of **nonimmunocompromised** (normal) humans elicits a mononucleosis illness that is clinically indistinguishable from EBV-mononucleosis. The majority of human infections are subclinical (with no overt symptoms) but may lead to life-long latent infection. CMV has a 4-8 week incubation period. Less common clinical sequelae include pneumonia, hepatitis, myocarditis, and meningoencephalitis.
   b. Illness in **immunosuppressed patients** [cancer, transplant (especially kidney), chemotherapy-induced, and AIDS patients] is often due to the reactivation of previously acquired infection. Infection in the immunosuppressed is more severe and is marked by fever, adenopathy, leukopenia, hepatosplenomegaly, and myalgias. Interstitial pneumonia, hepatitis, and gastrointestinal ulceration may also

CLINICAL CORRELATE

Due to the severe splenomegaly, patients with mononucleosis may undergo splenic rupture during physical exam.

IN A NUTSHELL

Epstein-Barr virus:

- *Herpes virus*
- *Causes infectious mononucleosis*
- *Hairy oral leukoplakia in immunocompromised patients*
- *Associated with Burkitt's lymphoma and nasopharyngeal carcinoma*
- *Heterophile antibodies; Monospot test*
- *Atypical lymphocytes*

arise in immune compromised individuals. **CMV retinitis** is seen in AIDS patients.

c. **Congenital disease** arises when the fetus acquires CMV virus transplacentally, causing **cytomegalic inclusion disease.** Infection can be acquired during any trimester. A severe fatal form of the illness is characterized by large intranuclear inclusion bodies in salivary glands, kidneys, brain, liver, and lungs. Periventricular calcifications are seen in the CNS; the infant will be deaf and have hemorrhagic cutaneous lesions (blueberry muffin baby).

d. **Antiviral therapy** is instituted in severe disease with **ganciclovir**. Mononucleosis in normal patients is usually self-limiting without antiviral treatment.

6. **Human herpesvirus-6** (HHV-6) is a lymphotrophic human herpesvirus. It is thought to be the etiologic agent of pediatric "sixth disease," or **roseola infection** (exanthem subitum).

D. **Poxviruses**

1. **Characteristics.** Poxviruses are the largest of all the viruses. They are linear **double-stranded DNA enveloped** viruses that **replicate entirely in the cytoplasm of infected cells.** They appear ovoid or brick-like in shape. The virion contains several enzymes for replication, including both DNA and RNA polymerases.

2. **Variola virus (smallpox virus)** is confined to humans and is spread by direct person-to-person contact. Smallpox is now officially considered eradicated after years of worldwide vaccination.

3. **Molluscum contagiosum** causes umbilicated **wart-like skin lesions** and satellite nodules on the periphery of the parent nodule. In children, the lesions are more commonly found on the trunk, face, or limbs. The sexually transmitted virus will be expressed as genital lesions.

   a. The disease is typically benign and self-limiting but may require up to 3 years to resolve (particularly genital lesions). The lesions may be numerous in immunocompromised (especially AIDS) patients.

4. **Cowpox** is an occupational disease transmitted to humans by contact with infected cow udders. Infection is usually restricted to fingers and hands and is self-limited. The disease is usually milder in vaccinated individuals.

E. **Hepadnaviruses** are DNA viruses that include the **hepatitis B virus (HBV),** which is discussed in the separate section below on hepatitis viruses.

F. **Parvoviruses** are small single-stranded DNA viruses. **Serotype B19** is the only human pathogen in the family. It causes **erythema infectiosum** ("Fifth disease") in children (characterized by slapped cheek rash), aplastic crisis in individuals with chronic hemolytic diseases such as thalassemia and sickle cell (the virus infects premature RBCs and kills them), and fetal infections that may cause hydrops fetalis or stillbirth of fetuses that are profoundly anemic.

NOTE

Other erythemas are measles, rubella, scarlet fever, roseola, and exanthem subitum (HHV-6).

RNA VIRUSES

A. **Picornaviruses** are small **(+) single-stranded RNA** viruses with a **naked** nucleocapsid. The RNA genome is **positive sense** (can serve as mRNA) and replication of picornaviruses occurs in the cytoplasm of the host cell. The picornaviruses can be divided into enteroviruses (poliovirus, coxsackie A and B, echovirus, and enterovirus) and rhinoviruses. Enteroviruses are acid-resistant and are able to survive in the GI tract; rhinoviruses are acid-sensitive.

1. **Polioviruses** bind to receptors in the gut and on neurons. Disease occurs only in primates, with the majority of infections expressed as subclinical disease.
   a. **Transmission.** Poliovirus is excreted in the feces and transmission occurs primarily by person-to-person contact and via contaminated water sources.
   b. **Pathogenesis.** Poliovirus is ingested, replicates in oropharyngeal and intestinal mucosa, and drains to the cervical and mesenteric lymph nodes. Transient viremia ensues and the virus then spreads systemically. CNS involvement may lead to the destruction of motor neurons in the spinal cord, resulting in flaccid paralysis.
   c. **Prevention.** Live attenuated virus vaccine (oral; OPV; Sabin) or the killed virus vaccine (IPV; Salk vaccine) are used to induce immunity. Both vaccines induce serum antibodies; only the oral vaccine induces gut immunity and sIgA synthesis.

NOTE

Polioviruses are polycistronic, which means that the RNA is read as large polyprotein messages (i.e., no stop codons in the genome). The individual proteins are produced by the action of a viral protease that "chops up" the polyprotein product of translation. Similar replicative processes occur in HIV; the newest AIDS drugs being used today are protease inhibitors.

CLINICAL CORRELATE

Polio vaccination schedule: currently IPV is given at the first two visits to the pediatrician (less than 4 months of age) together with DPT, Hib, and Hep B. This is followed by 2 doses of OPV. A booster may be given when starting school.

2. **Echoviruses** (Enteric Cytopathic Human Orphan) consist of 32 serotypes recognized by viral capsid antigen differences. All echoviruses infect the gastrointestinal tract although not all cause human disease.
   a. **Transmission and epidemiology.** Echoviruses are acquired by ingestion or inhalation. The initial infection occurs in the throat, followed by gastrointestinal tract infection. The incidence of clinical disease is increased in the summer months.

b. **Clinical manifestations.** Disease is observed as aseptic meningitis, fever, rash, enteritis, common colds, and/or acute hemorrhagic conjunctivitis. Less common symptoms include paralysis, pleurodynia, encephalitis, myocarditis, and respiratory illness.

3. **Coxsackieviruses** are divided into A and B groups according to their pathogenesis in suckling mice. **Coxsackie A** causes diffuse myositis of skeletal muscle; **Coxsackie B** causes focal necrosis of skeletal muscle and degeneration of brain and other tissues.
   a. **Transmission and epidemiology.** Epidemics occur in the summer and fall. Coxsackie is transmitted by nasopharyngeal secretions and by the fecal-oral route. Coxsackie infection is usually an asymptomatic or benign illness with the following exceptions.
   b. **Clinical manifestations**
      (1) **Coxsackie A** may cause **herpangina,** with headache, sore throat, dysphasia, stiff neck, fever, anorexia, and abdominal pain. Discrete vesicles are seen in the oropharynx. It is also the etiologic agent of hand-foot-and-mouth disease.
      (2) **Coxsackie B** may cause **myocarditis,** pericarditis, and **pleurodynia.**
      (3) Both viral groups may cause meningitis in humans.

4. **Enterovirus 72** is the etiologic agent of **hepatitis A.** It is discussed in the section on hepatitis viruses. Other enteroviruses are associated with exanthema and aseptic meningitis.

5. **Rhinoviruses** are associated most frequently with the **common cold.**
   a. **Transmission and epidemiology.** Rhinoviruses infect only human hosts and are commonly isolated from the nose and throat. The incubation period lasts 2-4 days, with clinical illness lasting up to a week. Over 100 serotypes have been identified, each conferring type-specific immunity.
   b. **Clinical manifestations** include upper respiratory tract irritation, headache, nasal discharge, cough, malaise, chills, and myalgia. Fever is usually limited or nonexistent, as is cervical lymphadenopathy.
   c. **Treatment and prevention.** Treatment is supportive. The large number of serotypes makes a vaccine impractical.

B. **Orthomyxoviruses** include **influenza viruses A, B, and C.** Orthomyxoviruses are medium-sized, negative-sense, **single-stranded, segmented RNA** with an **enveloped nucleocapsid.**

1. **Structure.** Influenza viruses are composed of eight separate segments of RNA.
2. **Classification** into types A, B, and C is based on NP protein and M protein antigens.
3. **Antigenic variation** is an important mechanism for the virus to evade immune system destruction, and is the reason for yearly change in vaccine composition.
4. **Transmission** is by inhalation. Epidemics occur with antigenic drifts while pandemics arise from antigenic shifts.
5. **Clinical manifestations.** Influenza type C causes symptoms of the common cold with an incubation period of 1-4 days. The symptoms of Influenza types A and B are more severe and include:
   a. Fever, chills, myalgia, and lassitude all occur abruptly
   b. Sore throat, headache, nasal congestion, and dry cough
   c. A potential progression to viral pneumonia or secondary bacterial infection (especially Staphylococcus). These symptoms usually occur in the elderly and debilitated.
   d. Influenza B is one of the many viruses associated with **Reye's syndrome.**
6. **Treatment and prevention**
   a. **Treatment** is effective only in type A infection and consists of **amantadine** or **rimantadine,** which decreases the duration of symptoms.
   b. **Vaccines** composed of inactivated virus are designed to elicit immunity against the existing serotypes in the population. Vaccines change from year to year based on the particular serologic determinants of the virus.

C. **Paramyxoviruses** are **negative-sense RNA** viruses with an **enveloped nucleocapsid.** Paramyxoviruses are genetically stable (no antigenic shifts or drifts). Initial infection is via the respiratory tract. These viruses are the most common cause of **respiratory infections in children.**

1. **Parainfluenza viruses** are ubiquitous and spread by aerosolized droplets. Parainfluenza is the etiologic agent of **croup**.
   a. Adults usually have neutralizing antibody to all four major serotypes. However, reinfection may occur despite the presence of neutralizing antibody. Reinfections are typically

mild compared to the primary infection, which usually occurs during the first 6 years of life.

b. **Clinical manifestations** are usually associated with febrile illness (viremia is uncommon). They include **laryngo-tracheobronchitis (croup),** or an obstruction due to swelling of larynx and trachea, **bronchiolitis** and pneumonia.

c. **Treatment.** Treatment is symptomatic; steam/nebulized air reduces symptoms.

CLINICAL CORRELATE

Children suffering from croup often have a characteristic "barking" cough.

3. **Measles virus (rubeola)** is a highly contagious childhood infection characterized by **fever and maculopapular exanthem.** Rubeola is transmitted by respiratory secretions. The virus multiplies in the oropharynx then spreads to lymphoid tissue followed by further viral replication throughout the reticuloendothelial system. Infection leads to permanent immunity since only one antigenic type exists.

a. **Clinical manifestations. Koplik spots,** bluish-white specks on a red base found on buccal mucosa, are pathognomonic for measles. Other clinical symptoms include an abrupt onset of anorexia, nausea, fever, malaise, coryza, conjunctivitis, and cough. A maculopapular, erythematous rash, lasting about 5 days, originates on the face and may spread to the torso.

NOTE

Koplik spots = measles = paramyxovirus

b. **Complications** of measles infection include **encephalomyelitis** (1 in 1,000 cases), pneumonia (in immunodeficient patients), otitis media, and **subacute sclerosing panencephalitis (SSPE).** SSPE is a fatal disease developing many years after acute infection. Measles infections are also thought to precede the onset of multiple sclerosis. These complications are thought to be due to an autoimmune response against nervous tissue that is induced by the measles virus.

c. **Treatment and prevention**

(1) **A live, attenuated vaccine** is available as part of the measles-mumps-rubella vaccine that is administered at **15 months** and again at **entry to school.**

NOTE

MMR vaccine is given at 15 months to avoid interference with viral replication by traces of maternal antibody.

(2) Unvaccinated children exposed to measles can be treated with pooled serum globulin since most donors have measles antibodies in their serum.

4. **Mumps virus** causes an acute contagious, nonsuppurative **parotitis** (either unilateral or bilateral). Less frequent clinical sequelae include **orchitis,** occurring in 20-35% of postpubertal males, and aseptic meningitis. However, as many as one-third of cases are asymptomatic. Mumps is prevented by **immunization with live attenuated virus** as part of the measles-mumps-rubella (MMR) vaccine.

5. **Respiratory syncytial virus (RSV)** is the primary cause of lower respiratory tract infections in **infants.**
   a. **Transmission** occurs via aerosolized droplets; it can also be spread by fomites.
   b. RSV replicates in the upper respiratory tract and persists in older children and adults. In infants and young children, the virus spreads to the lower respiratory tract, causing bronchitis and pneumonia.
   c. The spectrum of disease ranges from common cold-like symptoms to severe lower respiratory illnesses (especially in infants). Impaired immune functions will allow recurrent and prolonged infections with RSV.
   d. **Treatment** includes supportive care and **aerosolized ribavirin** in more severe cases. Virus-specific immunoglobulin injections are also being employed in the treatment of severe disease.

D. **Togaviruses** elicit diseases that range from febrile illness to encephalitis or severe bleeding disorders. Togaviruses contain **(+) single-stranded RNA** in an **enveloped nucleocapsid** with glycoprotein hemagglutinins in the envelope.

1. **Alphaviruses** multiply in many arthropods **(arthropod-borne)** and vertebrates. They are zoonotic agents spread to man by insect vectors. Alphaviruses include **encephalitis viruses**.
   a. **Eastern equine encephalitis (EEE)** virus causes a severe disease with 50-70% mortality. The illness is marked by the abrupt onset of headache, fever, and nuchal rigidity, with nausea, vomiting, and drowsiness. The individual may be left with neurologic deficits.
   b. **Western equine encephalitis** virus causes less severe disease and is seen more in children.

2. **Rubivirus** or **rubella** (3-day measles) is the virus causing German measles. It is the **only togavirus not transmitted by an arthropod vector.**
   a. **Rubella** consists of a single antigenic type that resembles measles. However, rubella is an infection of shorter duration and the disease is much less severe. The virus infects the upper respiratory tract and then spreads throughout the body via a viremia.
   b. A **morbilliform rash** occurs 2-3 weeks post-infection.
   c. **Congenital rubella.** Rubella virus may be **transmitted across the placental barrier.** This has serious consequences if it occurs in the first trimester. If the fetus survives, neurologic and other congenital abnormalities are common, including

IN A NUTSHELL

Paramyxoviruses:

- *Parainfluenza*
- *Measles*
- *Mumps*
- *RSV*

mental retardation, heart abnormalities, blindness, motor abnormalities, and encephalitis.

d. Live **attenuated virus vaccination** is effective for prevention of infection [as part of MMR (measles-mumps-rubella)].

E. **Flaviviruses** are very similar to togaviruses and specifically resemble alphaviruses. They are arthropod-borne viruses.

1. **Yellow fever** is a **mosquito-borne** flavivirus infection with an incubation period of 3-6 days.
   a. Two viral forms exist, urban (human reservoir) and jungle (monkey reservoir) types; both may result in severe disease affecting liver and kidney (with icterus and hemorrhage).
   b. Yellow fever is characterized by the acute onset of fever, jaundice, proteinuria, vomiting (vomitus is characteristically black) and the hemorrhage of internal organs with necrosis. Severe symptoms may lead to hypovolemic shock and death.
   c. A safe and effective attenuated vaccine (strain 17D) is available.

2. **Dengue fever** is also a **mosquito-borne** illness characterized by fever, rash, arthralgia, and lymphadenopathy. Only the more severe form involves hemorrhagic manifestations. Several antigenic serotypes are recognized and clinical complications may result in death (10% fatality rate). Dengue fever occurs primarily in the tropics.

F. **Bunyaviruses** are segmented **(–) single-stranded RNA** viruses with an **enveloped nucleocapsid.** Bunyaviruses are transmitted by **arthropod vectors (mosquitos)** and humans are only accidentally infected hosts.

1. **California encephalitis viruses** cause an abrupt fever and severe bifrontal headache. Other sequelae include CNS involvement requiring prolonged convalescence but with low morbidity and mortality.

2. **Hantavirus** causes hemorragic fever and acute respiratory distress syndrome with a high case fatality rate. They are natural pathogens of rodents. Humans become infected by inhalation of infectious urine or feces.

G. **Rhabdoviruses** include only one significant human pathogen, **Rabies virus.** Rabies is a **bullet-shaped** enveloped virus with **single-stranded (–) RNA.** It has a **nucleocapsid** with protruding glycoprotein spikes; the virus replicates in host cell cytoplasm. The rabies virus is of a single immunologic type eliciting neutralizing antibodies against the surface glycoproteins.

1. **Transmission** is dependent on the persistence of the virus in a wild animal reservoir (e.g., skunks, raccoons, bats, foxes), making this virus practically impossible to eradicate.

2. **Pathogenesis.** The virus enters through breaks in the skin produced from the **bite of a rabid animal.** The virus replicates in the muscle and connective tissue with an incubation period of 2-16 weeks. The virus moves through the axoplasm of peripheral nerves to the CNS, including the basal ganglia. Rabies may also inhabit the salivary glands and other tissues along its retrograde movement through the peripheral nerve pathways. **Negri bodies,** the cytoplasmic viral inclusions in neurons of the hippocampus, are pathognomic for the infection.

3. **Clinical manifestations.** The clinical course is described in four discrete phases: prodrome, sensory, excitement, and paralytic. Prodrome symptoms include paresthesia at wound site, irritability (including fever and a change in mood or temperment), and a flu-like illness. Pharyngeal spasms may arise, resulting in drooling (hydrophobia). Terminal disease involves seizures, coma, and, eventually, death.

4. **Diagnosis** is confirmed by the recovery of virus from saliva, by serology (direct immunofluorescence), or by immunofluorescence of Negri bodies in neural tissue (especially Ammon's horn).

5. **Treatment and prevention**
   a. **Vaccines** consist of inactivated virus from infected human diploid cells. Pets and high-risk individuals should be vaccinated, as should anyone with a history of being bitten by an animal that is possibly rabid.
   b. **Human rabies immunoglobulin (HRIG)** should be given immediately in cases of probable infection.

H. **Retroviruses** are diploid **(+) single-stranded RNA**, enveloped viruses associated with tumors, oncogenesis, and immunodeficiency disease (AIDS).

1. Retroviruses contain two copies of single-stranded RNA, and viral-encoded **reverse transcriptase** produces double-stranded DNA from the RNA.

2. The viral genome encodes three groups of proteins: **Pol protein** (reverse transcriptase and integrase), **Env protein** (type-specific envelope proteins), and **Gag protein** (type-specific viral core proteins).

NOTE

Negri bodies = rabies = rhabdovirus

3. This viral family includes **human T-cell leukemia viruses (HTLV I, II)** and **human immunodeficiency virus** (HIV). HTLV viruses are categorized as oncoviruses, while HIV is considered a lentivirus and is not oncogenic.

4. **Oncoviruses.** These viruses encode oncogenes that promote cell growth. The most important oncogenic retrovirus is **human T-lymphotropic virus 1 (HTLV 1)**. This virus infects CD4+ T cells (helper and delayed-type hypersensitivity). Infection can progress to **acute T-cell lymphocytic leukemia (ATLL)** (aka adult T-cell leukemia). **HTLV II** causes hairy cell leukemia.

5. **HIV is the etiologic agent of acquired immunodeficiency syndrome (AIDS).** AIDS was initially recognized as a clinical syndrome in 1981 followed by the isolation of HIV in 1983. HIV is a nononcogenic retrovirus that infects millions of people worldwide. HIV infects helper T cells via attachment to cell surface proteins (termed CD4), resulting in severe immunodeficiency. The virus also infects macrophages via an interaction with CD4 and CCR5 on the macrophage membrane. Patients are prone to opportunistic infections, malignancies, and wasting syndromes. Total CD4 T-cell counts are directly correlated with the degree of disease and infection. Opportunistic infections typically occur when the CD4 count drops below 200.

6. **HIV is classified as a lentivirus** (nononcogenic, cytocidal, retrovirus). HIV has a cylindrical, conical capsule that contains two positive, single-stranded RNA genomes with a glycoprotein envelope. The **gp120 glycoprotein spike** protrudes from this envelope and is the ligand for the CD4 molecule. Reverse transcriptase enzyme, critical to its replication, is inside the virion particle. The envelope proteins demonstrate considerable variation from virion to virion. This property makes it difficult for immune responses to clear the virus and complicates the development of a vaccine. Besides HIV, the lentivirus subfamily also includes SIV (simian), FIV (feline), and BIV (bovine) immunodeficiency viruses. Although this viral family shares common features (such as genes for reverse transcriptase), these viruses are infectious only in their respective species.

BRIDGE TO ANTIMICROBIALS

Treatment options for HIV are discussed in the chapter on Antimicrobial Agents later in this book.

BRIDGE TO IMMUNOLOGY

HIV is discussed in detail in the Clinical Immunology chapter of this book.

NOTE

The high level of antigenic variation among the HIV proteins is the result of inaccuracies in the action of reverse transcriptase, leading to incorporation of incorrect nucleotides.

HEPATITIS VIRUSES

Hepatitis viruses include both DNA (hepatitis B) and RNA (hepatitis A, C, D, E, and G) viruses.

A. **Hepatitis A virus (HAV)** is a **picornavirus.**

1. **Transmission.** Hepatitis A is transmitted **fecal-orally** after a 15-to-40-day incubation period. Hepatitis A is associated with epidemic and endemic spread of infection (usually occurring as familial or institutional outbreaks). Most childhood infections are asymptomatic, but adult disease can be severe.
2. There are typically no extrahepatic manifestations and there is **no chronic hepatitis or carrier state.** Infection is not associated with either cirrhosis or hepatic carcinoma. Fulminant hepatitis occurs only in 1-4% of clinical infections.
3. **Diagnosis** is made by the presence of **anti-hepatitis A IgM.**
4. There is a killed virus vaccine against hepatitis A that is recommended for individuals traveling to endemic areas, food handlers, and day-care workers. Pooled gamma globulin can be administered to travelers if there is insufficient time for active immunization.

B. **Hepatitis B virus (HBV)** is an **enveloped double-stranded DNA virus** with single-stranded segments. It is classified as a **hepadnavirus.**

1. Characteristic viral antigens include:
   a. **Surface antigen (HBsAg)** is found on the surface of the virion. Its presence in serum **indicates active viremia** (infectivity).
   b. **Core antigen (HBcAg)** is found in the capsid.
   c. **E antigen (HBeAg)** is another epitope found in the capsomere proteins.
2. **Antibodies to HBV**-specific antigens are diagnostic markers of disease activity.
   a. **Antibodies to the surface antigen (HBsAg)** are considered protective antibodies and are detected after the disappearance of the virus.
   b. **Antibodies to HBcAg** are not protective. They are detected just after the appearance of HBsAg and are used to confirm infection when HBsAg and anti-HBsAg are absent (window phase).

BRIDGE TO GASTROINTESTINAL

Hepatitis is also reviewed in detail in the Gastrointestinal Pathology chapter of NDB Pathology Notes.

NOTE

Hepatitis B vaccine is standard prevention for all health care workers exposed to blood and body fluids in their normal work. Doses are usually: initial, 1 month and 6 months (approximately). The recombinant DNA yeast source of the vaccine ensures no possibility of exposure to other viruses from the vaccination.

c. **Antibodies to HBeAg** are associated with a low risk of infectivity.

3. **Transmission** is parenteral or sexual. The majority of affected patients recover from the illness.

4. **Clinical manifestations** include: anorexia, nausea, vomiting, headache, fever, abdominal pain, dark urine, and, sometimes, jaundice.
   a. Liver function tests will indicate **elevated transaminases, hyperbilirubinemia, and elevated alkaline phosphatase.**
   b. Pathology is observed as the accumulation of inflammatory cells and parenchymal necrosis in liver.
   c. Extrahepatic manifestations include arthralgia, arthritis, nephritis, and dermatitis.
   d. Ten to fifteen percent continue to carry the virus and may develop **chronic persistent hepatitis or chronic active hepatitis** with subsequent fibrosis, cirrhosis, and hepatocellular carcinoma.

5. **Prevention. Recombinant HBsAg vaccine** is available and is recommended for all individuals at risk (e.g., health care workers). It is also now part of the protocol for **childhood immunizations.** Children should receive three doses: one at birth (up to 2 months), one at 2-4 months, and a final dose at 6-18 months. Children born of hepatitis B carriers should be injected at birth with hepatitis B-specific immunoglobulins (HBIG).

C. **Hepatitis C virus (HCV)** is a **positive-sense, single-stranded RNA virus** classified as a **flavivirus**. It is associated with **post-transfusion hepatitis**. HCV has been characterized as the etiologic agent for the majority (>50%) of non-A–non-B (NANB) hepatitis infections. Screening of blood for HCV has significantly reduced the incidence of transfusion-related hepatitis. HCV develops a chronic carrier in nearly 50% of patients and is associated with cirrhosis and hepatocellular carcinoma.

D. **Hepatitis D (delta agent)** is a defective RNA virus that can replicate only in cells concurrently infected with hepatitis B. This virion **requires the presence of hepatitis B** enzymes to replicate. It occurs in Italy and in the Near East.

E. **Hepatitis E** is caused by a single-stranded RNA virus that is similar to Norwalk agent (calicivirus). Clinically, it causes a disease similar to hepatitis A, but it can become fulminant in pregnant women (20% mortality). It is spread via fecal-oral route. It occurs primarily in the Far East.

NOTE

Not surprisingly, yet another hepatitis virus was recently identified (1996). Hepatitis G (HGV) is an RNA virus associated with both acute and chronic hepatitis. It has a global distribution and is transmissible by transfusion. It appears to be distantly related to hepatitis C.

Virus	Hep A	Hep B	Hep C	Hep D	Hep E
Genome	+RNA Picornavirus	DNA Hepadnavirus	+RNA Flavivirus	-RNA Delta agent	+RNA Calicivirus
Transmission	Fecal-oral	Parenteral Perinatal Sexual	Parenteral	Parenteral	Fecal-oral
Fulminant course	Rare	Rare	Rare	Frequent	In pregnant women
Chronicity	Never	Often	Very often	Very often	Never
Oncogenic	No	Yes	Yes	?	No

Table 11-1. Summary of hepatitis viruses.

Mycology

Fungi are eukaryotic organisms that can be classified as either yeast or molds based on their morphology and their mode of reproduction. The simplest way to review medically important fungi is to divide them into groups based on their clinical presentation: cutaneous mycoses, subcutaneous mycoses, systemic mycoses, and opportunistic mycoses.

INTRODUCTION

A. **Morphology.** Fungi are eukaryotes possessing a cell wall (composed of glucose and mannose polymers called **chitin**) and a cell membrane (containing **ergosterol**). Capsules are found only with *Cryptococcus neoformans*. The fungi are divided into yeasts or molds based on shape and mode of reproduction.

1. **Yeasts** have round or oval morphology and reproduce by budding.
2. **Molds** have tubular structures called **hyphae**. Molds grow by branching and longitudinal extension to form **mycelial structures.** Hyphae can be either **septate** (divided into nucleated cells) or **nonseptate** (coenocytic).
3. **Dimorphic fungi** grow in the host as a yeast-like form, but grow as molds at room temperature *in vitro*.

B. **Reproduction** can be sexual and/or asexual. **Asexual spores** form through mitosis. This form of reproduction is referred to as an "imperfect" state. Most pathogenic fungi are found only in the imperfect state.

C. **Immunity.** The T-cell response is protective in fungal diseases; antibodies are not, although they may have some role in serodiagnosis.

DERMATOPHYTOSIS (CUTANEOUS MYCOSES)

Dermatophytosis is a mycotic infection of any keratinous structure of the **skin and its appendages** by **Trichophyton (T.), Microsporum (M.), or Epidermophyton (E.)**. Candida can cause cutaneous infections but will be discussed later under Opportunistic Mycoses.

A. **Stratum corneum infections** are characterized by location. **Ringworm** or **tinea** is another name for these cutaneous mycoses. The clinical syndrome includes scaling associated with pruritus.
   1. **Tinea corporis** – body
   2. **Tinea cruris** – groin (jock itch)
   3. **Tinea pedis** – feet (athlete's foot)
   4. **Tinea manuum** – hands
   5. **Tinea capitis** – scalp

B. **Infections of the hair** include **tinea capitis** (scalp) and **tinea barbae** (beard). These infections are commonly due to organisms from the genera Trichophyton or Microsporum. **Ectothrix** is when fungi are present outside of the hair shaft. **Endothrix** is the term to describe fungi within the hair shaft.

C. **Infections of the nail** are primarily due to **tinea unguium.** ***Trichophyton rubrum*** and ***T. mentagrophytes*** are the two most common etiologic agents.

D. **Diagnosis** is made by microscopic examination of skin scrapings using **KOH,** by fungal cultures, or by UV (Woods) lamp examination.

E. **Treatment** consists of topical imidazoles such as **miconazole or clotrimazole** (only for stratum corneum infections) or oral griseofulvin. Tolnaftate may also be used for skin infections.

CLINICAL CORRELATE

The major dermatophyte that spreads from person to person is M. audouinii. M. audouinii and M. canis are the two species that fluoresce in UV light.

SUBCUTANEOUS MYCOSES

Subcutaneous mycoses typically result from implantation of the organism by means of some sort of trauma.

A. **Sporotrichosis** is caused by the dimorphic saprophytic fungus ***Sporothrix schenckii***. Sporotrichosis has worldwide distribution with all ages and both sexes affected. *S. schenckii* is isolated from soil, living plants, or plant debris. It is classically associated with **rose thorns** and is often called **"rose gardener's disease."**
   1. **Clinical disease** is generally limited to the skin and regional lymphatics **(lymphocutaneous sporotrichosis)**.

NOTE

If a question mentions roses, there is a very good chance the answer is Sporothrix schenckii.

B. **Mycetoma** (Madura foot, maduromycosis) is a local chronic progressive destruction of skin, subcutaneous tissue, fascia, muscle, and bone.

1. Transmission is by soil contamination of a wound. Lesions usually occur on the **feet or hands**.
2. Infection results in a suppurative granuloma with multiple sinus tracts, and **mycotic grains** (granules) are extruded.
3. Mycetoma is caused by infection with several fungi **(eumycotic mycetoma)** or by higher bacteria (actinomycotic mycetoma).

SYSTEMIC MYCOSES

Systemic mycoses are also called deep mycoses. They can invade organs and are potentially life threatening.

A. **Histoplasmosis** (Darling's disease) is caused by *Histoplasma capsulatum*, which is found in **bird and bat droppings**.

1. The diphasic pathogen *H. capsulatum* exists in soil in the mycelial phase and converts to the yeast phase at 37°C.
   a. The mycelial form has septate branching hyphae-bearing spores at the lateral or terminal positions.
   b. **Microconidia** spores are the infectious particles.
   c. **Macroconidia** (8-14 mm) develop **finger-like appendages** over their surfaces (tuberculate macroconidia spores), which is a morphology used to identify this species.
2. Yeast forms are found within macrophages.
3. **Transmission** is mediated by **airborne inhalation of microconidia spores** that get deposited in alveoli and spread through lymphatics to the regional lymph nodes. Hematogenous spread may lead to metastatic foci of infection in the liver, spleen, etc. Human-to-human spread is infrequent. Within 7-21 days of primary exposure, cell-mediated immunity develops with resulting **granulomas** at infected sites.
4. **Clinical manifestations** include **acute and chronic pulmonary infections** that very rarely progress to a disseminated histoplasmosis.
   a. **Acute pulmonary histoplasmosis** arises 5-21 days after exposure and is expressed as **headache and fever** in most or all cases. No treatment is usually required since it is a benign and self-limited disease.
   b. **Chronic pulmonary histoplasmosis** occurs in patients with chronic obstructive lung disease. It begins as a benign segmental interstitial pneumonitis with 20% of cases progressing to a chronic cavitary disease. Cavitary disease is treated

with a full course of **amphotericin B**. In some cases, surgical resection is necessary.

c. **Disseminated progressive histoplasmosis** is uncommon, occurring in persons with deficient cell-mediated immunity, chronic underlying debilitating disease, or in patients undergoing corticosteroid or immunosuppressive therapy.

(1) **Clinical manifestations.** A variety of clinical manifestations may arise, including systemic symptoms (fever, chills, anorexia, malaise, weight loss), hepatosplenomegaly (abnormal liver function tests), interstitial pneumonitis, and adrenal or renal involvement.

(2) **Treatment** consists of **amphotericin B.**

B. **Coccidioidomycosis** (San Joaquin fever; Valley fever) is due to infection with another dimorphic fungus, ***Coccidioides immitis.*** The organism is indigenous to deserts of the **Southwestern U.S.** and **northern Mexico.** *C. immitis* is inhaled as airborne arthroconidia (associated with fresh diggings, dust storms, etc.). Wind causes the spores to become airborne (infections increase during the dry dusty season).

1. **Clinical manifestations** arise as acute and/or potentially chronic and disseminated infection.

a. **Acute pneumonitis** is subclinical in 60% of cases and is detected only by skin testing. 40% develop an influenza-like syndrome 7-28 days following exposure.

(1) These symptoms include fever, malaise, dry cough, eosinophilia, toxic erythema (a fine, generalized erythematous macular rash), and erythema nodosum (particularly in females).

(2) **Treatment** is typically not required in adults since the pneumonitis will resolve spontaneously. Amphotericin B is given to infants, debilitated patients, or those at risk for dissemination; miconazole is given as an alternative.

b. **Disseminated coccidioidomycosis** occurs in less than 1% of infected patients.

(1) Patients at risk include leukopenic or immunosuppressed individuals, those with Hodgkin's or AIDS, and certain racial/ethnic groups (African Americans, Filipinos).

(2) Disseminated coccidioidomycosis may spread to any organ, causing a toxic state and high fever.

(3) **Treatment** consists of amphotericin B. Fluconazole may also be effective.

C. **Blastomycosis** is caused by ***Blastomyces dermatidis,*** a dimorphic fungus growing as a mycelial form at room temperature and as

yeast form at 37°C. Conidia spores are thought to be infectious for humans, converting to the yeast form after inhalation into the lungs. Infection occurs in normal hosts, usually in occupations associated with soil contact.

1. **Acute blastomycosis** (pneumonitis) can be asymptomatic or a severe, often fatal, illness. The clinical presentation is marked by an influenza-like syndrome with fever, chills, productive cough, and pleuritic chest pain.
   a. **Therapy** may not be required since acute blastomycosis is usually a benign, self-limited disease.
2. **Chronic blastomycosis** has a variable course of progressive illness. Lungs and skin are most commonly involved. Treatment consists of amphotericin B or ketoconazole combined with surgical excision or drainage of the local lesion.

OPPORTUNISTIC MYCOSES

The opportunistic fungi are those that cause disease in immunocompromised patients (AIDS, chemotherapy, transplants). Unlike the systemic mycoses, these fungi are sometimes normal flora and are not limited to certain geographic regions.

A. **Candida**. Candidal infections may be cutaneous or systemic.
   1. ***C. albicans*** is the major pathogenic species.
   2. Candida are normal inhabitants of mucocutaneous body surfaces, soil, hospital environments, and some foods. *C. albicans* colonizes normal skin and most diseases are endogenous in origin. Occasional person-to-person spread occurs (e.g., newborn thrush).
   3. **Invasive disease** results from host alterations, leading to a change in the commensal status of the organism. Factors important in the invasiveness of Candida include predisposing illnesses (such as diabetes mellitus), damaged mucosal surfaces (such as those caused by indwelling catheters), depression of the immune status (as in immunocompromised individuals), and the use of steroids or antibiotics.
   4. **Clinical manifestations** depend on the site of infection.
      a. **Oropharyngeal infection** (includes **thrush**) is observed as discrete or confluent **white patches** on the tongue and buccal mucosa. Microscopic examination of scraping reveals pseudohyphae. Therapy consists of nystatin suspension, oral ketoconazole, or mycelex troches.
      b. **Vaginal infection** is frequently seen in diabetes mellitus, antibiotic therapy, and in pregnancy. It is associated with

NOTE

Candida found in your dental patients may indicate AIDS, other immunosuppression, recent antibiotic use or other etiologies.

thick yellow-white discharge and intense pruritus. Nystatin suppositories are beneficial. Imidazole drugs (topical or oral) are also therapeutic.

c. **Gastrointestinal colonization** causes disease in malnourished or immunocompromised persons, or in persons undergoing prolonged intra-abdominal surgical procedures. Clinical presentation may include diffuse ulcerative and erosive **esophagitis**, gastritis, or multiple superficial ulcerations of the small and large intestine. Stool and throat cultures may not be diagnostic because of frequent colonization by Candida. Therapy consists of nystatin, ketoconazole, and low-dose amphotericin B.

d. **Invasive, disseminated infection** occurs, particularly in patients with leukemia, lymphoma, and AIDS. The GI tract is probably the most common portal of entry. Symptoms include fever, shock, hypotension, and prostration. Renal infection occurs usually from hematogenous spread and may result in renal failure. Endocarditis occurs in drug abusers.

e. **Chronic mucocutaneous candidiasis** represents extensive cutaneous disease that is refractory to treatment and can be disfiguring.

5. **Diagnosis** is made by demonstration of fungal pseudohyphae in the tissue or by culture and biochemical identification (e.g., urease positive).

6. **Treatment.** Systemic treatment includes ketoconazole or fluconazole; amphotericin B is used less frequently. Flucytosine is also valuable in systemic disease. Cutaneous involvement usually responds to topical miconazole.

B. **Cryptococcosis** is due to infection with ***Cryptococcus neoformans***. *C. neoformans* is an encapsulated yeast that reproduces by budding. *C. neoformans* is found worldwide in avian feces (particularly pigeon droppings), in soil, fruits, milk, and wood products. Immunosuppression due to malignancy or AIDS predisposes to this disease.

1. **Pulmonary disease** is common since this is the primary portal of entry. Disease is usually transient and not severe if the patient is otherwise healthy.

2. **Disseminated disease** arises most often in immunocompromised individuals.

a. **Central nervous system** involvement includes **meningitis** lesions that occupy the cerebral white and gray matter space.

NOTE

Think cryptococcus if:

- *Immunocompromised patient*
- *Meningeal signs*

b. **Treatment** consists of **amphotericin B** alone or in combination with **5-fluorocytosine.**

C. **Aspergillosis** is a disease arising from several species of ubiquitous molds. ***A. fumigatus*** is the most common species. Organisms are normal inhabitants of the soil, and spores are readily disseminated in the air.

1. **Allergic bronchopulmonary aspergillosis** is marked by a **hypersensitivity reaction** to the fungal antigens. Inhalation of conidia or mycelial fragments may elicit an IgE-mediated hypersensitivity reaction causing bronchospasm.
   a. **Clinical manifestations** include episodic wheezing, fixed or transient pulmonary infiltrates, fever, and peripheral **eosinophilia**.
   b. **Treatment.** No therapy is required if the disease is mild. Corticosteroids, however, are helpful in reducing symptoms.
2. **Aspergillomas (fungus balls)** are the result of colonization of pulmonary cavities (usually secondary to tuberculosis or sarcoidosis). Patients may be asymptomatic, but **hemoptysis** occurs in the majority of cases. Surgery is indicated for massive hemoptysis.
3. **Invasive aspergillosis** usually occurs as an opportunistic infection in immunocompromised patients, with iatrogenic neutropenia.
   a. **Pulmonary involvement** is present in 90-95% of cases. Invasive aspergillosis presents as an unremitting fever and pulmonary infiltrate despite therapy with broad-spectrum antibiotics. Necrotizing bronchopneumonia is common.
   b. Extrapulmonary dissemination to the esophagus, brain, or GI tract (with GI bleeding) occurs in about 25% of cases.
   c. **Diagnosis** must demonstrate tissue invasion. **Septate hyphae** will be seen in biopsy specimens.
   d. **Treatment.** Surgical removal of the aspergilloma may be necessary. Amphotericin B is the therapeutic standard, although itraconazole may be a valuable alternative.

D. **Zygomycosis (mucormycosis)** is most often caused by organisms from the genera **Rhizopus and Mucor**. These molds are ubiquitous on decaying vegetable matter in soil. They have **nonseptate hyphae.**

1. Individuals predisposed to invasive zygomycosis disease include those with **diabetic ketoacidosis**, leukemia, and lymphoma, antibiotic and steroid use, and infants and children with malnutrition.

IN A NUTSHELL

Fungi forms in vivo:

- *Coccidioides* → *spherules*
- *Histoplasma* → *intracellular yeast*
- *Blastomyces* → *broad-based buds*
- *Cryptococcus* → *large capsule*
- *Candida* → *pseudohyphae*
- *Aspergillus* → *branching septate hyphae*
- *Mucor/Rhizopus* → *nonseptate hyphae*

2. **Clinical manifestations**
   a. **Rhinocerebral disease** is the most common presentation. It typically occurs in **diabetics with ketoacidosis** and is an infection of the nasal mucosa, palate, sinuses, and/or orbit, whereby progressive neurological deficits ensue as the organism invades to the base of the brain.
   b. **Pulmonary disease** is usually the consequence of inhalation of spores in a patient with leukemia or lymphoma.
3. **Treatment** involves control of underlying disease such as diabetes, surgical debridement, and amphotericin B.

E. ***Pneumocystis carinii*** was originally thought to be a protozoa because of its morphological stages and sensitivity to antiprotozoal drugs. However, rRNA homologies suggest that it is a fungus.

1. **Clinical manifestations.** In individuals with normal cell-mediated immunity, infection is asymptomatic. Defects in cell-mediated immunity, such as AIDS, cause trophozoites to invade the alveoli and cause **interstitial pneumonia.**
2. **Treatment** for pneumonia consists of **trimethoprim-sulfamethoxazole** or **pentamidine.** Steroids are indicated for severe pneumonitis. Prophylactic and suppressive therapy consists of trimethoprimsulfamethoxazole, with dapsone or pentamidine as a second choice.

Protozoa

Protozoa are unicellular eukaryotic organisms. They have a true nucleus, multiple chromosomes, and organelles. All protozoa have endoplasmic reticulum and lysosomes, while some have mitochondria and Golgi apparatus. They usually reproduce asexually in the human host. Only those species significant for NDB background have been included.

INTRODUCTION

Protozoa are classified into four groups.

A. **Sarcodina**—amoeba

B. **Sporozoa**—sporozoans

C. **Mastigophora**—flagellates

D. **Ciliata**—ciliates

INTESTINAL AND MUCOCUTANEOUS PROTOZOA

Intestinal and mucocutaneous protozoa include *Giardia lamblia*, *Entamoeba histolytica*, *Trichomonas vaginalis*, *Isospora belli*, and *Cryptosporidium parvum*. Of these organisms, *Giardia lamblia* and *Entamoeba histolytica* have the simplest life cycles. The environmental form is called the **cyst.** Transmission of these organisms is fecal-oral, and results from the ingestion of contaminated water or food. Once in the intestine, the organisms excyst and the intestine is colonized by the motile, feeding **trophozoite.** This form is responsible for the pathology associated with disease. *Trichomonas vaginalis* is a sexually transmitted flagellated organism that undergoes binary fission. It has no cyst stage. *Isospora belli* and *Cryptosporidium parvum* both have complex life cycles that include sexual and asexual stages.

NOTE

While it behaves like a protozoa, pneumocystis is now classified as a fungus. It was discussed in the Mycology chapter.

NOTE

You should suspect Giardia in campers or hikers who present with diarrhea, bloating, flatulence, etc.

NOTE

Giardia trophozoites have a characteristic "face-like" appearance.

CLINICAL CORRELATE

The liver abscess is characterized by hepatomegaly, right upper quadrant pain, fever, and weight loss.

NOTE

All 3 protozoan discussed (giardiasis, amebiasis, Trichomonas vaginitis) are treated with metronidazole.

NOTE

On the exam, you are most likely to see Cryptosporidium as a cause of diarrhea in AIDS patients.

A. ***Giardia lamblia*** is the infectious agent of **giardiasis,** acquired by the ingestion of fecal-contaminated water or food.

1. Manifestation of *G. lamblia* colonization ranges from asymptomatic infection to inflammation with **diarrhea, cramps, bloating, flatulence,** malaise, weight loss, and occasional steatorrhea from impaired fat absorption.

B. ***Entamoeba histolytica*** is the etiologic agent for **amebiasis.** *E. histolytica* is spread by the fecal-oral route and is more common in areas with poor sanitation.

1. **Clinical manifestations**. The organisms infect the colon of humans, invading and lysing intestinal epithelium with subsequent ulceration.
   a. Clinical disease may be mild with diarrhea, abdominal cramps, nausea, vomiting, and flatulence.
   b. More severe (invasive) disease results in **dysentery,** severe abdominal pain, dehydration, and **bloody stools.**
   c. Severe infection can also result in perforation of the bowel wall, resultant peritonitis, and **liver abscess formation.**

C. ***Trichomonas vaginalis*** causes **vaginitis** in women. Infection in men is frequently asymptomatic but may lead to prostatitis or urethritis.

1. **Clinical manifestations** in women are dependent upon the physiological status of vaginal pH, flora, and the intensity of the infection. Symptoms include dyspareunia, dysuria, pruritis, and a **copious discharge** that is **yellow and frothy.** Symptoms worsen when vaginal pH is more alkaline.

D. ***Cryptosporidium parvum*** is acquired by ingestion of oocysts. The oocysts release sporozoites that differentiate into trophozoites. The trophozoites attach to epithelial cells mainly in the jejunum and ileum.

1. **Clinical manifestations**. Cryptosporidium is increasingly recognized as a cause of traveler's and seasonal diarrhea. Disease presents with watery diarrhea, anorexia, and weight loss that clear in 10 days to 2 weeks. However, in immunodeficient patients, especially those with **AIDS,** it can be life-threatening chronic disease.
2. **Treatment**. Currently, no effective treatment exists for Cryptosporidium.

BLOOD AND TISSUE PROTOZOA

Blood and tissue protozoa include *Plasmodium* species, *Bab*

pecies, *Leishmania* species, *Trypanosoma* species, and *Toxoplasma gondii*.

A. **Plasmodium** are intracellular parasites with a complex life cycle. The **sexual phase** occurs in the **Anopheles mosquito** and is transmitted to humans by the bite of the female; the **asexual phase** occurs in **humans. Malaria** affects 200 to 400 million people per year and kills approximately 1% of them. It is mainly a tropical disease. However, in the United States, it is the most common imported acute febrile illness.

1. **Malaria in humans** is caused by one of four species of Plasmodium.
2. **Clinical manifestations.** All species can cause anemia, dehydration, electrolyte abnormalities, splenomegaly, and acute splenic rupture.
   a. **Periodic fever and chills** arise and last up to one hour followed by diaphoresis. Nausea, anorexia, vomiting, and malaise are clinical complications that follow.
   b. *P. falciparum* causes the greatest morbidity and mortality due to the greater degree of parasitemia and adherence to vascular endothelium. Complications can include the following:
      (1) **Blackwater fever** characterized by intravascular hemolysis, icterus, hemoglobinuria, and acute renal failure.
      (2) **Cerebral malaria** characterized by headache, confusion, rapid coma, and convulsions. Mortality from cerebral malaria is between 20 and 30%.
3. **Treatment** depends on the stage of the illness and the infecting organism.
   a. **Chloroquine** is the treatment of choice unless the infecting organism is chloroquine-resistant *P. falciparum.*
   b. Alternate drugs for chloroquine-resistant *P. falciparum* include quinine, quinidine, mefloquine, pyrimethamine, and artemisinin.
4. **Risk factors.** Genetic factors influence host susceptibility to malarial infection.
   a. In West Africa, heterozygotes for the sickle cell trait have a selective advantage because the growth of the trophozoite is inhibited by low oxygen tension.
   b. Glucose-6-phosphate dehydrogenase (G6PD) deficiency increases resistance to *Plasmodium falciparum* infection.

B. ***Babesia microti*** is an erythrocytic protozoan that is responsible for the human disease **babesiosis.**

1. **Transmission and epidemiology**. *Babesia microti* is transmitted via *Ixodes dammini*, the same tick vector as in Lyme disease. *Babesia* primarily infects animals; humans are incidental hosts. The species has a rodent reservoir. Babesiosis is common in the **Northeastern United states,** especially on islands off the Northeast coast (e.g., Nantucket). Babesiosis causes severe disease or death if the patient is **asplenic.**

C. **Leishmania** species cause zoonotic infections that are transmitted by the bite of the phlebotomine **sandfly.** Leishmaniasis occurs primarily in India, Africa (Old World), and Central and South America (New World). The parasite invades the host's reticuloendothelial cells and resides in the phagolysosomes.

1. **Clinical disease** may be expressed as cutaneous, mucocutaneous, or visceral forms.
2. **Treatment** of cutaneous leishmaniasis depends upon the location and extent of the lesion. Pentavalent antimonials can be used to treat large, disfiguring lesions. Visceral and mucocutaneous leishmaniasis are treated with the pentavalent antimonials sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime).

D. **Trypanosoma** is the species of protozoan responsible for African and American **trypanosomiasis.**

1. **African trypanosomiasis, African sleeping sickness,** is caused by *Trypanosoma brucei gambiense* in Western and Central Africa and by *Trypanosoma brucei rhodiense* in Eastern Africa. Both species are transmitted by the bite of an **infected tsetse fly,** and lead to CNS involvement and death if not treated.
   a. **Clinical manifestions**
      (1) Disease is first expressed as a **chancre** appearing at the site of inoculation.
      (2) Parasitemia arises 2-3 weeks later, invading the lymphoid-macrophage system and leading to fever, rash, headache, lymphadenopathy, and mental status changes.
      (3) Once the organism spreads to the CNS , the disease progresses with anorexia, lassitude, fatigue, wasting, and eventually stupor, coma, and death.
   b. **Treatment.** Infection is difficult to cure. **Suramin** is the drug of choice for treatment of patients with hemolymphatic disease, but with normal CSF. The drug of choice for disease with CNS involvement is **melarsoprol.**
2. **Chagas' disease** or American trypanosomiasis, is caused by ***Trypansoma cruzi.*** It is a zoonotic infection transmitted by

NOTE

If you see "Nantucket" – think babesiosis.

IN A NUTSHELL

Review of the tick-borne diseases:

- *Babesiosis (Babesia)*
- *Lyme disease (Borrelia)*
- *Endemic relapsing fever (Borrelia)*
- *Rocky Mountain spotted fever (Rickettsia)*
- *Ehrlichosis (Ehrlichia)*
- *Tularemia (Francisella tularensis)*

NOTE

Worldwide, Chagas' disease is one of the most common causes of heart disease.

Reduviid bug or "kissing bug." The organism enters through the mucous membranes or breaks in the skin.

a. **Clinical disease** is caused by the invasion of the lymphoid-macrophage system, endocrine glands, myocardium, and neural tissue.
b. **Treatment** of acute disease consists of **nifurtimox,** a derivative of nitrofuran. No effective therapy exists for chronic disease.

E. ***Toxoplasma gondii,*** the etiologic agent of **toxoplasmosis,** occurs worldwide.

1. There are three forms of the organism: the **trophozoite,** which is the invasive form; the **tissue cyst,** which contains intracystic organisms; and the oocyst in which the sporozoites are formed.
2. **Transmission** to humans usually occurs via secondary hosts.
   a. The organism undergoes the sexual cycle in the intestine of cats, the definitive host, to form oocysts that are passed in the feces.
   b. Ingested oocysts invade the intestine of intermediate hosts and disseminate hematogenously to form pseudocysts in tissue.
   c. Cysts can occur in all tissues, including muscle, brain, and eye.
   d. Tissue cysts can then be ingested by humans in raw or undercooked meat from intermediate hosts.
   e. Alternatively, infection can be acquired from ingestion of the oocysts in cat feces.
3. **Clinical manifestations.** Intact cell-mediated immunity usually restricts infection to the asymptomatic pseudocyst stage in adults.
   a. Primary infection may be associated with a mild mononucleosis-like illness.
   b. In **immunodeficient patients** (AIDS, treated cancer patients), disease usually arises as an acute infection originating from a chronic, quiescent infection that is activated due to altered immunity. CNS disease may be expressed as a meningoencephalitis or as a **mass lesion** with seizures.
   c. **Congenital infection** may result if the mother acquires primary infection during early pregnancy. **Chorioretinitis,** diffuse intracranial calcifications, hydrocephaly, anemia, and seizures are associated with congenital disease.
4. **Treatment** consists of **pyrimethamine** and **sulfadiazine** if the disease is severe.

NOTE

If you see an HIV/AIDS patient with "ring enhancing lesions" on MRI, think toxoplasmosis. (These same lesions in a 72-year-old smoker → think metastatic lung cancer.)

NOTE

Toxoplasma is the "T" in the ToRCHeS acronym for congenital infections:

- *Toxoplasma*
- *Rubella*
- *CMV*
- *HSV, HIV*
- *Syphilis*

Antimicrobial Agents

This chapter reviews the drugs used to treat the pathogenic microorganisms described in the previous chapters. The outline is divided into antibacterials (antibiotics), antimycobacterials, antifungals, and antiviral agents. For each drug, the mechanism of action, indications for use, and side effects and toxicity are reviewed. Note, however, that this chapter is an overview. Only those areas directly related to NDB1 are covered. More detailed knowledge is required for NDB2 Pharmacology questions.

ANTIBACTERIAL AGENTS

Antibiotics are chemicals that may be produced entirely by microorganisms or that may be modified (semisynthetic) to broaden the spectrum of activity, increase the chemical stability, or improve the pharmacokinetic properties. Some antibiotics inhibit bacterial growth (bacteriostatic); others kill organisms (bactericidal); and some possess both properties in a dose-dependent manner. Antibiotics are usually classified according to bacterial specificity or mechanism of action.

A. Overview of classes of antibiotics

1. **Cell wall synthesis inhibitors.** Bactericidal agents that interfere with the synthesis of bacterial cell walls make microorganisms vulnerable to changes in the osmolarity of the environment. The cell wall contains complex, cross-linked peptidoglycans, which conveys rigidity. The cross-linking occurs across peptide chains as a result of transpeptidation and is catalyzed by enzymes inhibited by antibiotic.
   a. All **beta-lactam antibiotics** include β-lactam rings:
      (1) **Penicillins and cephalosporins.** These drugs act as analogs of D-alanyl-D-alanine. They prevent the final step in cell wall synthesis by **inhibiting the transpepti-**

dase enzyme responsible for cross-linking and peptidoglycan synthesis. The penicillins and cephalosporins differ primarily in that the penicillins are derivatives of 6-aminopenicillanic acid, whereas the cephalosporins are characterized by substituent groups added to 7-aminocephalosporanic acid. Differences in susceptibility of Gram-positive and Gram-negative organisms depend on structural differences in cell walls, the presence of different binding proteins, the nature of peptidoglycans, and the activity of autolytic enzymes.

(2) **Carbapenems**

(3) **Monobactams**

b. The drugs listed below inhibit cell wall synthesis at early stages within cell cytoplasm; drugs must penetrate the cell membrane to be effective.

(1) **Vancomycin** blocks the growing end of peptidoglycan.

(2) **Bacitracin** blocks dephosphorylation of lipid carrier.

(3) **Cycloserine** prevents D-alanine additions to form pentapeptides.

2. **Protein synthesis inhibitors.** These drugs affect the function of bacterial ribosomes, thereby inhibiting protein synthesis.

a. The protein synthesis inhibitors that interact with the 30S ribosomal subunit include the following:

(1) **Aminoglycosides** are bactericidal agents that block initiation of protein synthesis, causing accumulation of protein synthetic initiation complexes. Because tetracyclines do not inhibit bacterial cell wall synthesis, they are effective against cell wall-deficient organisms and bacterial variants that may develop during treatment with cell wall-inhibiting antibiotics.

(2) **Tetracyclines** act as bacteriostatic agents that inhibit binding of aminoacyl-transfer RNA (tRNA) to mRNA ribosome complex.

(3) **Spectinomycin** blocks initiation.

b. The protein synthesis inhibitors that interact with the 50S ribosomal subunit are:

(1) **Erythromycin** is a macrolide antibiotic that contains large lactone ring to which sugars are attached. Erythromycin inhibits bacterial protein synthesis by blocking release of the uncharged tRNA from the 50S ribosomal subunit. It is prescribed for patients allergic to penicillins.

(2) **Clindamycin** is a lincosamide that has an unknown mechanism thought to be similar to that of erythromycin (inhibits 50S subunit of ribosome). While clindamycin's Gram-positive antibacterial spectrum is sim-

IN A NUTSHELL

Cell wall synthesis inhibitors:

- *Penicillins*
- *Cephalosporins*
- *Carbapenems*
- *Monobactams*

} *β-lactams*

- *Vancomycin*
- *Bacitracin*
- *Cycloserine*

IN A NUTSHELL

Protein synthesis inhibitors:

- *Aminoglycosides*
- *Tetracyclines*
- *Spectinomycin*

} *30S*

- *Erythromycin*
- *Lincomycin*
- *Clindamycin*
- *Chloramphenicol*

} *50S*

to that of erythromycin, it has broader coverage, including anaerobes.

(3) **Chloramphenicol** is a bacteriostatic agent that acts by binding the 50S subunit of the bacterial ribosome to inhibit peptidyltransferase action. This binding is readily reversible and is inhibited by the macrolide and lincosamide antibiotics.

3. **Antimetabolites. Folate antagonists** are bacteriostatic agents that interfere with bacterial synthesis or reduction of folate.
   a. **Sulfonamides** arrest cell growth by inhibiting the bacterial synthesis of folic acid. Sulfonamides are **structural analogs of the folic acid precursor, para-aminobenzoic acid (PABA)**. They competitively inhibit dihydropterate synthetase, the enzyme that directs the incorporation of PABA and a pteridine moiety into dihydropteroic acid. Organisms that do not synthesize folic acid because they obtain it from other sources (e.g., humans) are unaffected.
   b. **Trimethoprim** is a structural analog of the pteridine portion of **dihydrofolate reductase** and acts as a **competitive inhibitor** of this enzyme, which converts dihydrofolate to tetrahydrofolate (active form of folic acid). Tetrahydrofolate is required as a methyl donor in the synthesis of adenine, guanine, and thymine.
4. **Cell membrane inhibitors** act directly on the cell membrane to affect permeability and lead to leakage of intracellular compounds.
   a. Cell membrane inhibitors that disrupt cell membranes of Gram-negative bacteria (bactericidal) include:
      (1) **Polymyxin,** which binds to phospholipids and alters cell permeability
      (2) **Colistin**
   b. Cell membrane inhibitors that interact with membrane sterols in fungal cells include:
      (1) **Amphotericin B,** which binds to ergosterol-altering cell membranes.
      (2) **Nystatin** (same mechanism as amphotericin)
      (3) **Fluconazole, clotrimazole,** and **ketoconazole** inhibit ergosterol synthesis.
5. **Nucleic acid synthesis inhibitors**
   a. **Fluoroquinolones** (e.g., ciprofloxacin) and **nalidixic acid inhibit DNA gyrases** (topoisomerases) necessary for supercoiling of DNA.
   b. **Rifampin** binds to and **inhibits DNA-dependent RNA polymerase** present in bacteria.

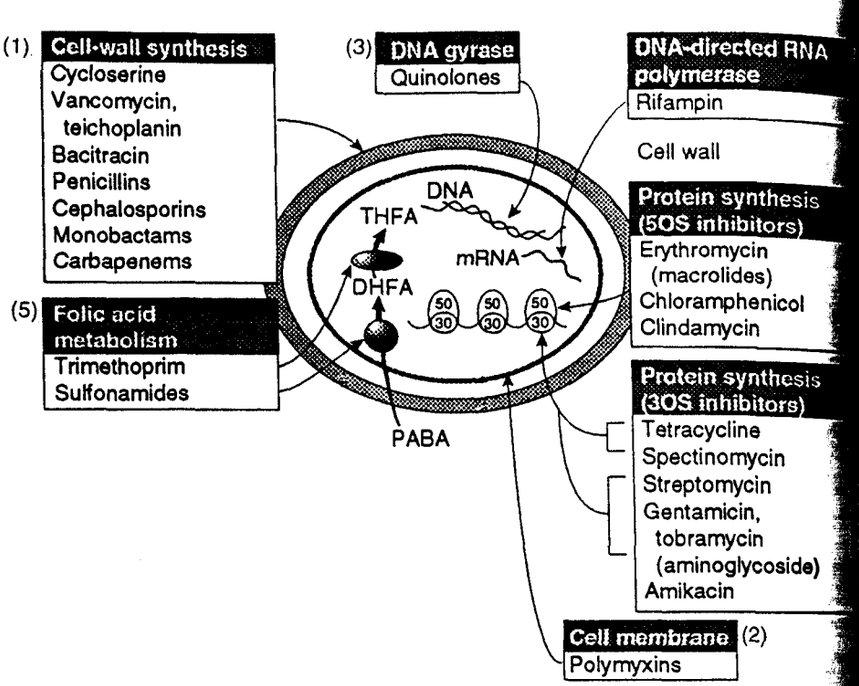


Figure 14-1. Antimicrobial sites of bactericidal action on microorganisms. The five mechanisms are: (1) inhibit cell wall synthesis; (2) damage cell membrane; (3) modify nucleic acid/DNA synthesis; (4) modify protein synthesis; and (5) modify metabolism within the cytoplasm. (THFA = tetrahydrofolic acid; DHFA = dihydrofolic acid; and PABA = para-aminobenzoic acid.) (Modified with permission from Brody™, Larner J, Minneman KP, Neu HC: Human Pharmacology: Molecular to Clinical, 2nd ed. St. Louis, Missouri, Mosby-YearBook, 1994, p 618.)

B. **Efficacy**

1. Successful antimicrobial therapy depends on achieving inhibitory or bactericidal activity at the site of infection without significant toxicity to the host.
2. In most infections, the normal local and systemic host defense mechanisms play a crucial role in the final elimination of the pathogen.
3. The degree of *in vitro* susceptibility of bacterial strains to particular antibacterial agents is estimated by determining the **minimum inhibitory concentration (MIC),** the lowest concentration of antibiotic that prevents growth, and the **minimum bactericidal concentration (MBC),** the lowest concentration of antibiotic that kills all the organisms in an *in vitro* MIC assay.

C. **Resistance.** Microorganisms are capable of acquiring resistance to antimicrobial agents by genetic changes that are passed on from generation to generation.

NOTE

The disk diffusion method is commonly used to determine antibiotic sensitivity. The organism is exposed to disks saturated with different antibiotics. After growth, the degree of growth inhibition around each disk is measured and the susceptibility/resistance to the antibiotic is determined.

1. **Spontaneous chromosomal mutations** produce a genetically altered bacterial population that is resistant to the drug action, survives, and gives rise to a new drug-resistant population.
2. Drug resistance is usually acquired, not by chromosomal change, but by R-factors from other bacteria in the form of extrachromosomal DNA pieces that contain resistance-mechanism information. R-factors are **plasmids** that carry genes for resistance to one or more antibiotics. Plasmid transfer accounts for over 90% of antibiotic resistance.
3. **Changes in drug permeability.** Tetracyclines are able to accumulate in susceptible bacteria; in resistant bacteria, resistance occurs by increasing the energy-dependent efflux of tetracyclines.
4. **Drug deactivation.** The principal mechanism of resistance to the penicillins and cephalosporins is by **β-lactamase** action. Aminoglycosides and chloramphenicol are inactivated by acetylation or other enzymatic modification.
5. **Decreased drug conversion to active compound**. The antifungal drug, flucytosine, must be converted *in vivo* to fluorouracil, which is further metabolized to the active form of the drug. Fungi become resistant to flucytosine by losing enzyme activity along the activation pathway.
6. **Altered metabolic pathway** may occur in bacteria resistant to sulfonamides and in fungi resistant to flucytosine. Some sulfonamide-resistant bacteria can use preformed folic acid (e.g., from mammalian cells).
7. **Altered amount of drug receptor.** Some organisms become resistant to penicillins and cephalosporins by synthesizing altered penicillin-binding proteins and to fluoroquinolones by altered DNA gyrase activity.
8. **Decreased receptor affinity for drug.** Resistance to erythromycin may be associated with alteration of a specific protein on the 50S subunit of the bacterial ribosome necessary for drug binding.
9. Evaluations of the effectiveness of antimicrobial agents must use the results of clinical trials as well as the *in vitro* activity of the drug against potential pathogens. Combinations of antibiotics are often used to broaden coverage with mixed or unknown types of infection, to prevent or delay the emergence of bacterial resistance to the drugs, and to achieve therapeutic synergy.

BETA-LACTAMS AND OTHER CELL WALL SYNTHESIS INHIBITORS

A. **Penicillins** are β-lactam antibiotics similar to the cephalosporins. The development of large-scale production has led to structural modifications of the original penicillin G, with formation of derivatives with greater effectiveness against a variety of infections.

1. **Penicillin G (benzyl penicillin)**
   a. **Pharmacologic properties**
      (1) They possess bactericidal action that affects only growing cells during cell wall synthesis.
      (2) Gastric acid hydrolyzes penicillin G and only 30% of the active drug is absorbed. It is not normally taken orally.
      (3) Crystalline penicillin G given intramuscularly results in therapeutic peak plasma levels that last for 2-3 hours. It may be given intravenously when large doses are required.
   b. **Indications for use**
      (1) **Gram-positive cocci,** including *Streptococcus pneumoniae* and *Streptococcus pyogenes*
      (2) **Gram-negative cocci,** including *Neisseria meningitidis* and *Neisseria gonorrhoeae* that do not produce β-lactamase
      (3) **Gram-positive bacilli,** including *Bacillus anthracis, Clostridium perfringens, Listeria monocytogenes,* and *Corynebacterium diphtheriae*
      (4) **Treponema pallidum** is the causative agent of syphilis.
   c. **Side effects and toxicity** of penicillin are generally few, but in some patients, allergic reactions may occur.
      (1) **Hypersensitivity reactions** occur in up to 10% of patients. Symptoms include rashes, urticaria, fever, serum sickness, Stevens-Johnson syndrome, and anaphylaxis due to antibody formation to degradation products. Cross-sensitivity with cephalosporins also exists.
      (2) Diarrhea
      (3) Jarisch-Herxheimer reaction (flu-like symptoms, including fever, chills, and myalgia) can occur in secondary syphilis within the first few hours following penicillin G therapy.
   d. **Resistance mechanisms**
      (1) Bacteria containing **penicillinase enzymes (β-lactamases)** in their periplasmic space can inactivate penicillin G by opening the β-lactam ring. It is a common resistance mechanism among Staphylococci and Gram-negative

bacteria. **Methicillin-resistant organisms** (e.g., many *Staphylococcus epidermidis*) are resistant even to penicillins not sensitive to penicillinase; these organisms are usually treated with **vancomycin.**

(2) Penicillin penetrates the bacterial cell envelope and attaches to a number of penicillin-binding proteins (PBPs) on the bacterial cytoplasmic membrane. Bacterial resistance may also result from altered affinity and number PBPs.

2. **Penicillin V**

a. **Pharmacologic properties.** It is effective against oral bacteria; most used penicillin in dentistry. It is available only in oral form. It is more resistant to gastric acid destruction and has greater gastrointestinal absorption than penicillin G.

b. **Indications for use**

(1) Penicillin V is less active than penicillin G, especially against Gram-negative bacteria (e.g., Neisseria, meningococcal meningitis).

(2) It is used only when an oral form is desired for susceptible organisms.

3. **Broad-spectrum penicillins**

a. **Ampicillin**

(1) **Pharmacologic properties** are similar to penicillin G (both are destroyed by β-lactamase), but ampicillin is acid stable and has increased activity against Gram-negative organisms. It is available for oral and parenteral administration. Resistance mechanisms include inactivation by penicillinase or altered properties of PBPs.

(2) **Indications for use** include some gonococcal infections, upper respiratory infections (e.g., *H. influenzae, S. pneumoniae, S. pyogenes*), urinary tract infections (e.g., *E. coli,* Enterococcus, *Proteus mirabilis*), meningitis (e.g., *H. influenzae, S. pneumoniae, N. meningitidis*), and Salmonella and Shigella infections.

b. **Amoxicillin** is a parahydroxyl derivative of ampicillin.

(1) **Pharmacologic properties** are similar to ampicillin, but there is better intestinal absorption and less gastrointestinal disturbance. Amoxicillin is hydrolyzed by β-lactamases but is stable in combination with β-lactamase inhibitor, **clavulanic acid (Augmentin)**. Amoxicillin is resistant to gastric acid destruction and can be taken orally. It attains higher peak serum levels than ampicillin after similar oral dosage; this is the major difference between the two agents.

(2) **Indications for use** include infections of the skin, soft tissue, and lower urinary and respiratory tracts, caused by

NOTE

Penicillin V was the drug of choice for dental procedure antibiotic prophylaxis of subacute bacterial endocarditis prior to 1990. It was replaced by amoxicillin in 1990 due to the greater gastric absorption of amoxicillin. As of 1997, the standard regimen for prophylactic premedication is 2.0 gm of amoxicillin 1 hour before procedure, with no follow-up dose.

nonpenicillinase-producing strains of Staphylococci, Streptococci, *H. influenzae*, *E. coli*, and *P. mirabilis*; uncomplicated anogenital and urethral gonococcal infections; and otitis media.

c. **Amoxicillin plus clavulanic acid (Augmentin)**

(1) **Pharmacologic properties** are similar to amoxicillin, but the combination with clavulanic acid broadens coverage to include β-lactamase-producing organisms, including *H. influenzae*. Augmentin is administered orally only.

(2) **Indications for use** include severe otitis media, sinusitis, pneumonia (e.g., *H. influenzae, Moraxella catarrhalis*), and animal bites (*Pasteurella multocida*).

d. **Ampicillin with sulbactam (a penicillinase inhibitor)**

(1) **Pharmacologic properties** are similar to ampicillin. Sulbactam broadens coverage to include β-lactamase-positive organisms and some anaerobes. There is parenteral administration only.

(2) **Indications for use** include intra-abdominal infections where anaerobic coverage is desired and severe urinary tract infections (UTIs), including those caused by Enterococci.

NOTE

Clavulanic acid and sulbactam are inhibitors of β-lactamase that can greatly broaden the spectrum of penicillins.

4. **Antipseudomonal penicillins include**
   a. **Carbenicillin**
   b. **Ticarcillin**
   c. **Ticarcillin with clavulanic acid (Timentin)**
   d. **Piperacillin**
5. **Penicillinase-resistant penicillins (antistaphylococcal penicillins) include**
   a. **Methicillin**
   b. **Oxacillin, dicloxacillin, cloxacillin**
   c. **Nafcillin**

B. **Cephalosporins** originated from a Cephalosporium fungus and have been found to inhibit *aureus*. As with the penicillins, semisynthetic cephalosporins now exist for clinical use and are classified as first-, second-, or third-generation drugs.

1. **Mechanism of action** is similar to that of the penicillins; cephalosporins block terminal cross-linking of the bacterial cell wall peptidoglycan and activate cell wall autolytic enzymes.
2. The nucleus of the cephalosporins consist of a β-lactam ring fused to a six-membered ring similar in structure to the nucleus of the penicillins.
3. Resistance to the cephalosporins is by β-lactamases and by lack of antibiotic penetration in Gram-negative organisms.

4. The **first-generation cephalosporins** are broad-spectrum agents with activity against Gram-positive organisms (except Enterococcus and methicillin-resistant Staphylococcus) and Gram-negative bacteria (including *E. coli,* Klebsiella, Proteus).
5. Anaerobic bacteria, including *Bacteroides fragilis,* are sensitive to the **second-generation agents** (e.g., cefoxitin), which are less potent than first-generation cephalosporins but have broader Gram-negative coverage.
6. The **third-generation** drugs can **penetrate the central nervous system (CNS)** and, with the exception of cefoperazone, are active against bacterial meningitis.
7. **Pharmacologic properties** are similar to the penicillins but somewhat less potent, requiring higher dosages.
   a. Most cephalosporins are excreted by the kidney (glomerular filtration and tubular secretion).
   b. Those with acetylated residues (cephalothin, cephapirin) are metabolized in the liver, and their metabolites are renally excreted.
   c. Some are highly protein bound (e.g., cephalothin, cefamandole, cefoxitin, and cephapirin).
   d. Variable susceptibilities to β-lactamases
8. **Indications for use** include upper respiratory infections, acute otitis media, urinary tract infections, skin and soft tissue infections with *S. aureus,* prophylaxis before surgery, systemic infections with bacteria sensitive to these agents, and life-threatening infections before specific organisms are identified (due to broadness of antimicrobial spectrum).
9. **Side effects and toxicity**
   a. Allergic reactions may occur, including urticaria, rash, fever, and eosinophilia. There is a 5%-10% cross-reactivity between cephalosporins and penicillins in those hypersensitive to penicillins.
   b. There may also be nausea, vomiting, diarrhea, and elevated liver function test values; pseudomembranous colitis).
   c. **Hypoprothrombinemia** (with cefamandole, cefoperazone, moxalactam)
   d. **Disulfiram-like reaction** with alcohol (with cefamandole, cefoperazone, moxalactam)
   e. Nephrotoxicity
   f. Positive direct Coombs' test

IN A NUTSHELL

- *1st generation → Similar to penicillin and ampicillin*
- *2nd generation → Extended Gram-negative coverage, including anaerobes*
- *3rd generation → Excellent Gram-negative coverage; cross blood-brain barrier*

10. **Specific agents** (Table 15-1)

Drug	Route and bacterial specificity
Note: Cephalosporins are similar to the penicillins in structure; they also have similar activities, which have increased with each "generational" modification in structure.	
First generation	
Cephalothin Cefazolin Cephalexin Cephradine Cefaclor	These drugs inhibit most Gram-positive organisms except Enterococci. They are used to treat respiratory infections in children.
Second generation	
Cefamandole	This drug is more active than first-generation drugs against *Haemophilus* species, some *E. coli*, *Klebsiella*, and other Enterobacteriaceae.
Cefuroxime	This drug inhibits Gram-positive organisms, is excellent against *Haemophilus* and *Neisseria* species, and has greater resistance to β-lactamase than does cefamandole.
Cefoxitin	This drug is less active against Gram-positive organisms, but its high β-lactamase stability and inhibitability to Enterobacteriaceae and 85% of anaerobic bacteria make it useful for aspiration pneumonitis and intra-abdominal and intrapelvic infections.
Third generation	
Cefotaxime Ceftizoxime	These drugs are excellent against Gram-positive Streptococci, including *S. pneumoniae* and *Haemophilus* and *Neisseria* species.
Ceftriaxone	This drug is similar in action to cefotaxime and ceftizoxime. It is used to treat nosocomial infections, Lyme disease, and gonorrhea.
Cefixime (oral agent)	It precipitates in the bladder and may cause diarrhea. Cefixime inhibits Streptococci, *Haemophilus* species, *Neisseria*, *Moraxella*, and many Enterobacteriaceae. It is used to treat respiratory infections.
Cefoperazone	Cefoperazone has an Antabuse-like action and it changes prothrombin activity. It is less β-lactamase stable but is active against *Pseudomonas*.
Fourth generation	
Cepirome Cefepine	These drugs have increased activity against Gram-positive bacteria, inhibit *Pseudomonas* species, and are not labile to some β-lactamases.

Table 15-1. Cephalosporins.

C. **Monobactams: aztreonam.** As the name implies, monobactams have a single β-lactam ring.

1. **Pharmacologic properties**. Aztreonam interferes with cell wall synthesis. Excretion is mostly urinary. Tissue levels are excellent

D. **Carbapenems: imipenem**

1. **Pharmacologic properties**
   a. Imipenem is a synthetic β-lactam antibiotic.
   b. It inhibits cell wall synthesis.

E. **Other cell wall inhibitors**

1. **Vancomycin**
   a. **Pharmacologic properties**
      (1) Vancomycin is a bactericidal agent that inhibits cell wall synthesis by binding D-Ala-D-Ala, thus preventing its incorporation into peptidoglycan.
      (2) It is poorly absorbed after oral administration.
      (3) It is active against **Gram-positive bacteria** but not against Gram-negative organisms.
   b. **Indications for use**
      (1) Vancomycin is given orally to treat **pseudomembranous colitis** caused by the anaerobe *Clostridium difficile.*
      (2) It is given intravenously for treatment of **methicillin-resistant Staphylococci** and **penicillin-resistant Pneumococci**.
      (3) It is useful in patients allergic to penicillins and cephalosporins in the treatment of Gram-positive infections.
      (4) Finally, vancomycin is used with gentamicin for *Streptococcus faecalis* or *Streptococcus viridans* endocarditis or for serious infections in patients with penicillin allergy.
   c. **Side effects and toxicity**. Rapid infusion can cause facial and neck erythema ("red man" syndrome). There may also be ototoxicity (rare), phlebitis, and nephrotoxicity.
2. **Bacitracin** is a cyclic polypeptide that inhibits cell wall synthesis. Because of nephrotoxicity, use is limited to **topical application.**
3. **Cycloserine** is a broad-spectrum antibiotic. Its use is limited to treatment of **tuberculosis (TB).**

NOTE

Vancomycin is used in dental antibiotic prophylaxis in some penicillin-allergic patients.

IN A NUTSHELL

Uses of vancomycin:
- *Methicillin-resistant Staphylococci*
- *Enterococcal infections in the penicillin-allergic patient*
- *Pseudomembranous colitis (C. difficile)*
- *Penicillin-resistant S. pneumoniae*

PROTEIN SYNTHESIS INHIBITORS

A. **Aminoglycosides**

1. **Overview.** Binds the 30S ribosomal subunit and causes change in the codon:anticodon recognition sites. **Bactericidal action** results from the irreversible inhibition of the initiation of protein synthesis.

a. **Pharmacologic properties**. All aminoglycosides are **administered parenterally** because of poor intestinal absorption and are used against **aerobic Gram-negative** bacteria.

b. **Side effects and toxicity**

(1) **Ototoxicity and renal impairment** are side effects of all aminoglycosides and are usually dose dependent, while hypersensitivity reactions are idiosyncratic.

(2) Resistance develops rapidly to all aminoglycosides mainly by plasmid-encoded enzyme inactivation.

2. **Gentamicin**

a. **Indications for use**

(1) Gentamicin is used for serious infections with susceptible Gram-negative bacteria.

(2) It is also used topically in burns infected with Pseudomonas and for ocular infections.

3. **Tobramycin**

a. **Indications for use.** Tobramycin is used with penicillins, cephalosporins, and alone to treat infections in all sites except the CSF.

4. **Streptomycin**

a. **Indications for use**

(1) Although primarily reserved for treatment of TB (*Mycobacterium tuberculosis*), it is active against many other microbes and is often used in combination with a β-lactam antibiotic in life-threatening diseases such as *K. pneumoniae* pneumonia.

(2) It may be used with penicillin to treat streptococcal endocarditis.

(3) Streptomycin is ineffective against anaerobic infections because there is an oxygen-dependent transport step required for its penetration into the cell.

b. **Side effects and toxicity**. Ototoxicity and nephrotoxicity are more common than with most other aminoglycosides.

5. **Neomycin**

a. **Indications for use**

(1) Neomycin is used as a **topical application** for superficial **skin infections.**

(2) It is given as an oral prophylaxis to **prepare the bowel** prior to intestinal surgery.

c. **Side effects and toxicity**. Neomycin is the most toxic aminoglycoside with dose-related ototoxicity and nephrotoxicity; **it is not for parenteral administration.** There may be diarrhea and malabsorption following oral administration.

NOTE

Tobramycin is the most active aminoglycoside against Pseudomonas.

6. **Amikacin** has the widest antibacterial spectrum of the aminoglycosides.
   a. **Indications for use.** Amikacin is used mainly for treatment of organisms resistant to other aminoglycosides. It is the drug of choice for burns infected with resistant Pseudomonas.

B. **Tetracyclines.** Tetracyclines (four rings) are broad-spectrum **bacteriostatic** antibiotics. They **bind at the 30S ribosomal subunit** and block protein synthesis by interfering with the interaction of aminoacyl-tRNA and the mRNA-ribosome complex.

1. **Tetracycline**
   a. **Pharmacologic properties**
      (1) Variable gastrointestinal absorption after oral administration occurs by forming insoluble complexes in the gut with calcium or other ions. The presence of food, milk, metallic salts, or antacids results in poor absorption.
      (2) Intravenous administration is used for serious infections, malabsorption syndromes, and critically ill patients.
      (3) The drug's broad antibacterial spectrum includes a wide variety of Gram-positive and Gram-negative bacteria.
      (4) Resistance to tetracycline develops in direct proportion to usage. Resistance mechanisms include tetracycline-resistant ribosomes, bacterial production of enzymes that degrade the antibiotic, and decreased permeability of the bacterial cell surface to the drug (plasmid mediated).
   b. **Indications for use**
      (1) Tetracycline is useful for the following infections: **Rickettsia** (Rocky Mountain spotted fever, Q fever), **Chlamydia** (lymphogranuloma venereum; psittacosis, *Chlamydia pneumoniae*), ***Francisella tularensis, Vibrio cholerae*** (cholera), **Borrelia** (Lyme disease), **Ureaplasma,** and ***Mycoplasma pneumoniae.***
      (2) Tetracycline is an alternative treatment for infections with *L. monocytogenes* and *N. gonorrhoeae.*
      (3) It is also used as a treatment for chronic **severe acne** (topical or oral administration).
   c. **Side effects and toxicity**
      (1) Intravenous administration can produce thrombophlebitis and hepatotoxicity.
      (2) Fetal and neonatal **tooth discoloration** make it contraindicated during pregnancy, nursing, and in children under 8.
      (3) Gastrointestinal disturbances, including esophageal ulceration, occur in 10% of patients.

IN A NUTSHELL

Tetracyclines can be used to treat the following organisms:

- *Rickettsia*
- *Chlamydia*
- *Francisella tularensis*
- *Vibrio cholerae*
- *Mycoplasma pneumoniae (2nd choice after erythromycin)*
- *Ureaplasma*
- *Borrelia*
- *Severe acne*

NOTE

Tetracycline staining in teeth is found histologically in dentin.

(4) Dry mouth, hoarseness, stomatitis, glossitis, pharyngitis, enterocolitis, and proctitis can occur.

(5) There can be hepatotoxicity with prolonged administration of high doses; pregnant women are more susceptible.

(6) Pseudotumor cerebri, elevation of blood urea nitrogen (BUN), and photosensitivity also occur.

2. **Doxycycline and minocycline**

a. **Pharmacologic properties** are like those of the naturally occurring tetracyclines. Resistance mechanisms are similar to other tetracyclines. Some species of *B. fragilis* are more susceptible to doxycycline and minocycline than to tetracycline.

b. **Indications for use**

(1) These drugs are commonly used to **treat sexually transmitted diseases;** they are very effective against both chlamydia infections and gonorrhea. They are also used in the treatment of Lyme disease.

c. **Side effects and toxicity** involve vestibular disturbances, including dizziness and nausea.

C. **Spectinomycin.** Spectinomycin inhibits protein synthesis by interacting with the 30S ribosomal subunit. Clinical use is limited to the **treatment of *N. gonorrhoeae*** in patients allergic to penicillin.

D. **Macrolides and lincosamides.** The antibacterial action of these agents is through binding to the **50S subunit** of the bacterial ribosome and interfering with protein synthesis.

1. **Erythromycin**

a. **Pharmacologic properties**

(1) Because most of the drug is inactivated by acid, it is administered orally with an enteric coating that dissolves in the duodenum. Most of drug is concentrated in the liver and excreted in bile.

(2) Erythromycin diffuses readily into all body fluids except the brain or CSF.

(3) Erythromycin has an antimicrobial spectrum similar to penicillin G and is active against Gram-positive bacteria including Listeria, *S. aureus, Strep. pneumoniae, Strep. viridans, Strep. faecalis,* Clostridium, Corynebacterium and Actinomyces.

(4) Erythromycin is active against *Mycoplasma pneumoniae,* Treponema, Chlamydia, *Rickettsia,* and *Legionella pneumoniae.*

(5) Resistance is now becoming more of a problem as nosocomial staphlococcal infections are now resistant. Mechanisms include failure of the organism to take

NOTE

Effective in 1997, erythromycin is no longer the recommended endocarditis prophylaxis for penicillin-allergic patients, and has been replaced by clindamycin, azithromycin or clarithromycin.

antibiotic and plasmid-encoded decreased binding to the 50S ribosomal subunit.

b. **Indications for use**

(1) Erythromycin is used in patients with penicillin allergy; it is an **alternative to penicillin** for susceptible pathogens.

(2) It is the drug of choice for the treatment of **Legionnaire's disease** and ***M. pneumoniae.***

(3) It is an alternative to penicillin in treating syphilis.

(4) Long-acting macrolides such as azithramycin and clarithramycin are replacing erythromycin as preferred therapeutics.

(5) It is an alternative to tetracycline in treating chlamydial infections.

(6) A topical preparation of erythromycin is used in the treatment of acne.

c. **Side effects and toxicity**

(1) Gastrointestinal disturbances are common.

(2) Like the aminoglycosides, sensorineural hearing loss may occur with large doses.

2. **Clindamycin**

a. **Pharmacologic properties**

(1) It is widely distributed to bones, fluids, and tissues but shows poor CNS penetration. The spectrum of activity is similar to that of erythromycin.

(2) It is the drug of choice for serious infections caused by the **anaerobic organisms,** *B. fragilis,* Fusobacterium, and Peptococcus.

(3) It is active against common Gram-positive pathogens, including Staphylococci and Streptococci, but it is not active against most Gram-negative organisms.

(4) Clindamycin reacts with the 50S ribosomal subunit and interferes with amino acid transfer to the growing peptide chain (mechanism unknown).

b. **Indications for use.** Clindamycin's most important use is in the treatment of severe anaerobic infections caused by Bacteroides and other anaerobes.

(1) Primary **lung abscesses** with susceptible pathogens and aspiration pneumonia

(2) **Intra-abdominal sepsis** and intrapelvic infections

(3) **Orthopedic infections** with susceptible pathogens

(4) Acne (topically)

c. **Side effects and toxicity**

(1) Clindamycin can produce **pseudomembranous enterocolitis**, now described as antibiotic-associated colitis (AAC), resulting from suppression of intestinal organ-

IN A NUTSHELL

Erythromycin is the first choice for treatment of Legionella and M. pneumoniae. It is a backup choice in the treatment of syphilis (penicillin is first choice) and chlamydia (tetracycline is first choice). It is also used widely as an alternative to penicillin in penicillin-allergic patients.

IN A NUTSHELL

Clindamycin:

- *Used to treat anaerobic infections (e.g., Bacteroides)*
- *Causes pseudomembranous colitis*

isms and proliferation of the anaerobe, ***Clostridium difficile.*** *C. difficile* is treatable with **vancomycin.**

E. **Chloramphenicol** was isolated from cultures of Streptomyces and is the first completely synthetic antibiotic.

1. **Pharmacologic properties**
   a. A potent inhibitor of microbial protein synthesis, chloramphenicol **binds to the 50S subunit** of bacterial ribosome to block the action of peptidyltransferase.
   b. Chloramphenicol is bacteriostatic for many bacteria and rickettsiae.
   c. It can be administered parenterally or orally with good CNS penetration.
   d. It is clinically active against many strains of Gram-positive and Gram-negative bacteria, Rickettsiae, anaerobes, and Mycoplasma.
   e. Gram-negative bacteria develop resistance via a factor acquired by conjugation.

2. **Indications for use**
   a. Chloramphenicol is used as a treatment for acute typhoid fever and other serious **Salmonella infections**, particularly in developing nations; infections with ampicillin-resistant *H. influenzae,* especially meningitis (some third-generation cephalosporins are also effective); and meningitis caused by *N. meningitidis* and *Streptococcus pneumoniae* in patients hypersensitive to penicillin.
   b. In general, use is limited by toxicity; it is used only for serious infections when other agents are not viable alternatives.

3. **Side effects and toxicity**
   a. Pancytopenia, which is dose-related and often reversible, may occur.
   b. Aplastic anemia may occur in 1/30,000 patients. It is not dose-related and may occur months after drug use.
   c. Hemolytic anemia may occur in patients with glucose-6-phosphate (G6PD) deficiency.
   d. Nausea, vomiting, glossitis, stomatitis, diarrhea, and enterocolitis may occur.
   e. **"Gray baby syndrome"** occurs, especially in premature infants of mothers on chloramphenicol due to an infant's immature liver function (lacking glucuronide synthetase); it is potentially fatal.

IN A NUTSHELL

Protein synthesis inhibitors:

Bind to 30S ribosomal subunit:
- *Aminoglycosides*
- *Spectinomycin*
- *Tetracyclines*

Bind to 50S ribosomal subunit:
- *Erythromycin*
- *Clindamycin*
- *Chloramphenicol*

ANTIMYCOBACTERIAL AGENTS

M. tuberculosis invades many organs and requires prolonged therapy. Because single-drug therapy for a long period (weeks) allows the growth of resistant mutants, TB must be treated simultaneously with **two or more drugs.** Standard therapy for pulmonary and extrapulmonary TB may be either isoniazid and rifampin for 9 months or isoniazid and ethambutol for 8 months. In cases of infection with drug-resistant TB, overwhelming disseminated TB (miliary), or TB meningitis, three-drug regimens are often used. Atypical Mycobacterium infections (e.g., *Mycobacterium avium intracellulare*) are usually treated with multidrug regimens. A summary of these agents is provided in Table 15-2.

A. **Treatment of tuberculosis.** The number of cases in the U.S. has increased dramatically in part due to immigration, AIDS, and the number of homeless individuals.

Drug	Adverse effects
Isoniazid	Elevation of hepatic enzymes, peripheral neuropathy, hepatitis, CNS effects, and increased phenytoin concentration
Rifampin	Orange-red discoloration of secretions, urine, tears, and contact lenses; hepatitis, drug fever, flu-like symptoms, and thrombocytopenia; interferes with methadone,warfarin, medroxyprogesterone, theophylline, dapsone, and ketoconazole
Pyrazinamide	Gastrointestinal upset, elevation of liver enzymes, rash, arthralgia, and hyperuricemia
Ethambutol	Optic neuritis (everything appears green), decreased visual acuity, and skin rash
Streptomycin	Ototoxicity, nephrotoxicity, hypokalemia, and hypomagnesemia
Ciprofloxacin	Abdominal cramps, gastrointestinal upset, insomnia, headache, photosensitivity, and hypersensitivity reactions; drug interactions with warfarin and theophylline
Amikacin	Auditory and renal toxicity, vestibular toxicity (rare), hypokalemia, and hypomagnesemia
Ethionamide	Gastrointestinal upset, bloating, liver enzyme elevation, metallic taste, and hypothyroidism (especially if on para-aminosalicylic acid [PAS])
Cycloserine	Psychosis, depression, seizures, rash, headache, and increased phenytoin concentrations
Para-aminosalicylic acid	Gastrointestinal upset, elevated liver enzymes, sodium loading, decreased digoxin, and increased phenytoin levels
Clofazimine	Orange-brown skin discoloration, gastrointestinal complaints, and rare visual disturbances
Dapsone	Anemia, rash, and methemoglobinemia

Table 15-2. Drugs used to treat tuberculosis and leprosy.

NOTE

Mycobacteria were discussed in detail in the Mycobacteria and Actinomycetes chapter of this book.

ANTIVIRAL AGENTS

Viruses are obligate intracellular parasites that depend on the metabolism of the host cell. Therefore, agents that inhibit or kill viruses are also likely to injure the host cells that harbor them. Developmental approaches in the design of antiviral agents have focused on the selective inhibition of enzyme systems unique to virus-infected cells. However, at this time, very few agents have been found to be both effective and safe.

A. **Treatment of herpesviruses**

1. **Acyclovir** requires phosphorylation to be effective. It is phosphorylated efficiently by a **virus-specific thymidine kinase** and becomes trapped within virus-infected cells.
   a. **Pharmacologic properties**
      (1) Acyclovir **inhibits multiplication of various herpesviruses,** including varicella-zoster, herpes simplex types I and II, and Epstein-Barr virus. It is **not** effective against cytomegalovirus (CMV).
   b. **Indications for use**
      (1) It is used as a topical treatment for herpes simplex infections with little skin penetration.
      (2) Intravenous treatment is required for mucosal, cutaneous, and disseminated herpes simplex infections and treatment of herpes zoster or chickenpox in immunocompromised hosts.
      (3) Oral administration can lessen the severity of genital herpes if administered early in the attack, but intravenous therapy is necessary for severe infections.
   c. **Side effects and toxicity**
      (1) Nephrotoxicity may occur.
      (2) Encephalopathy, bone marrow depression, and abnormal hepatic function may occur.
2. **Vidarabine,** also called **ara-A,** is an adenosine arabanoside that is phosporylated within cells and **inhibits viral DNA polymerases.**
   a. **Pharmacologic properties**
      (1) It inhibits viral DNA polymerase.
   b. **Indications for use**
      (1) It is used in the treatment of ocular herpes simplex keratoconjunctivitis.
      (2) It is used in the parenteral treatment of herpes simplex encephalitis (if acyclovir resistant).
      (3) It is used in the treatment of herpes zoster infections in patients with suppressed immunologic response.

3. **Ganciclovir**
   a. **Pharmacologic properties**. Ganciclovir is a synthetic analog of guanosine whose mode of action is similar to that of acyclovir; i.e., it **inhibits viral DNA polymerase** after being converted to the triphosphate form in the infected cell.
   b. **Indications for use**
      (1) Available only in intravenous form, ganiclovir is active against CMV and other herpesviruses.
      (2) It is primarily used for **CMV infections** in immunocompromised patients.
      (3) It is more toxic than acyclovir, so use is restricted to CMV.

NOTE

Ganciclovir → CMV

4. **Foscarnet**
   a. **Pharmacologic properties**. Foscarnet inhibits viral DNA polymerase.
   b. **Indications for use.** It is effective in **CMV retinitis** and herpesvirus infections that are resistant to ganciclovir and acyclovir, respectively.

NOTE

Unlike acyclovir and ganciclovir, foscarnet does not need to be activated by a viral kinase.

5. **Idoxuridine** (IdUR) is used only **topically.**
   a. **Pharmacologic properties**
      (1) As a thymidine analog, it can be used to treat herpes simplex keratitis and dendritic ulcers.

B. **Treatment of respiratory viral infections**

1. **Amantadine and rimantadine** are used for the treatment and prophylaxis of **influenza A.** They are ineffective against influenza B.
   a. **Indications for use**
      (1) Prophylactic administration of the drug to high-risk patients in the presence of an influenza A virus epidemic
      (2) Treatment shortens the duration of influenza symptoms in patients with influenza A if administered within the first 12-24 hours.
      (3) Amantadine is used in treating symptoms of **Parkinson's disease** because it causes the release of dopamine and other catecholamines from neuronal storage sites and delays reuptake of these neurotransmitters into synaptic vesicles.
   b. **Side effects and toxicity**
      (1) Common CNS reactions include irritability, tremor, slurred speech, ataxia, depression, insomnia, lethargy, and dizziness.

2. **Ribavirin** is a synthetic purine nucleoside analog active against **respiratory syncytial virus.**
   a. **Indications for use.** Aerosols are used to treat respiratory syncytial virus in infants and children.
   b. **Side effects and toxicity** include anemia and conjunctivitis.

IN A NUTSHELL

Antivirals:

• *Acyclovir →*	*Inhibits viral DNA polymerase. Used to treat HSV, VZV, and EBV.*
• *Vidarabine → (ara-A)*	*Inhibits viral DNA polymerase. Used primarily in invasive HSV infections resistant to acyclovir (e.g., encephalitis).*
• *Ganciclovir →*	*Inhibits viral DNA polymerase. Used to treat CMV infections.*
• *Foscarnet →*	*Inhibits viral DNA polymerase. Used to treat ganciclovir-resistant CMV retinitis in immunocompromised patients.*
• *Idoxuridine → (IdUR)*	*Inhibits enzymes involved in DNA synthesis. Used topically for treatment of herpetic keratitis.*
• *Amantadine, → Rimantadine*	*Interfere with Influenza A virus penetration and uncoating. Used in treatment and prophylaxis of Influenza A.*
• *Ribavorin →*	*Interferes with nucleic acid synthesis (DNA and RNA). Used to treat infections caused by respiratory syncytial virus. Also effective against Lassa fever.*
• *Zidovudine → (AZT)*	*Inhibits reverse transcriptase. Used in treatment of HIV.*
• *ddC, ddI, and → 3TC*	*Same mechanism as AZT. Used in AZT-resistant cases.*
• *Protease → inhibitors (ritonavir, saquinovir, indinavir)*	*Newer agents used in HIV treatment. Block viral protease required for normal viral protein synthesis.*
• *Interferons→ (α, β, γ)*	*Interfere with viral protein synthesis. Used in treatment of hairy cell leukemia, Kaposi's sarcoma, and condyloma acuminatum.*

C. **Treatment of human immunodeficiency virus (HIV).** Because antiviral drugs inhibit virus replication after infection has occurred, they are most effective when given at an early stage of infection. Current practice is to use these drugs when $CD4^+$ cell number drops below 400/μl or to use them as prophylaxis in individuals accidentally exposed to HIV or born of HIV^+ mothers.

1. **Zidovudine (AZT)**
   a. **Pharmacologic properties**
      (1) AZT is a thymidine analog that terminates DNA transcription by viral reverse transcriptase, preventing viral replication.
      (2) AZT penetrates the CNS and undergoes hepatic and renal excretion.
   b. **Indications for use.** AZT is used in the treatment of **AIDS and various stages of HIV infection** (studies suggest prolonged survival in HIV-positive patients).
   c. **Side effects and toxicity**. Side effects are numerous, but toxicity associated with **bone marrow suppression** is the most serious problem.
      (1) Anemia, which may be severe and require transfusions, can occur.
      (2) Megaloblastic erythrocyte changes and granulocytopenia may occur within weeks of therapy, but using lower doses of AZT decreases side effects.
      (3) Liver function abnormalities are exacerbated by acetaminophen, and bone marrow toxicity is exacerbated by ganciclovir.
      (4) Less severe adverse effects of AZT include headache, insomnia, diarrhea, rashes, and fever.
2. **Dideoxycytidine (ddC) and dideoxyinosine (ddI)** also inhibit reverse transcriptase. They have activity against AZT-resistant HIV. Both drugs cause peripheral neuropathies.
3. **3-thiacytidine (3TC)** is an inhibitor of reverse transcriptase, most often used in conjunction with AZT. Pancreatitis is a major adverse side effect, particularly in infants.
4. **Protease inhibitors** such as ritonavir, saquinovir, and indinavir are the newest drugs used in the treatment of HIV. They interfere with the viral protease that cleaves the polypeptide chain translated from the polycistronic viral mRNA, thus, the gag-pol polyprotein is not cleaved and functional reverse transcriptase is not produced. These drugs are usually used in conjunction with reverse transcriptase inhibitors. Adverse reactions commonly seen are diarrhea, abdominal discomfort, and nausea.

D. **Human interferons** are naturally occurring glycoproteins produced in response to viral infection. They inhibit viral replication and promote antiviral responses. Three types of interferon are produced: **interferon** α (produced by monocytes), **interferon** β (produced by fibroblasts), and **interferon** γ (produced by T lymphocytes).

1. **Pharmacologic properties**
   a. Once released, IFNs **inhibit viral multiplication in other cells** by inducing cellular enzymes that block translation of viral mRNA to viral proteins.
   b. The interferons have been produced by recombinant DNA techniques and can be generated in large amounts.
2. **Indications for use.** IFNs are used in the treatment of **hairy cell leukemia, Kaposi's sarcoma** in patients with AIDS; **condylomata acuminatum** (genital warts); and herpes keratoconjuctivitis, in combination with other antiviral agents.

ANTIFUNGAL AGENTS

A. **Polyene antibiotics.** Polyene antibiotics are effective against both filamentous and yeast-like fungi, including Histoplasma, Blastomyces, Coccidioides, Cryptococcus, Candida, Aspergillus, Mucor, and Rhizopus species. The polyenes have no activity against dermatophytes or bacteria. Polyenes interact with ergosterols in the cytoplasmic membrane of fungi, leading to rapid leakage of small molecules and fungal death.

1. **Amphotericin B**
   a. **Pharmacologic properties**
      (1) Systemic infections are treated by slow intravenous infusion; amphotericin B cannot be administered orally.
   b. **Indications for use**
      (1) Amphotericin B is a broad-spectrum antifungal agent used for treatment of **systemic fungal infections.** It is active against Histoplasma, Cryptococcus, Candida, Blastomyces, and Aspergillus.
      (2) The drug is also used in the treatment of **mucocutaneous leishmaniasis** and **amebic meningoencephalitis** (freshwater amebae).
   c. **Side effects and toxicity**
      (1) Low therapeutic index (small test dose usually given)
2. **Nystatin (mycostatin)**
   a. **Pharmacologic properties**
      (1) Its structure and mechanism of action are similar to amphotericin B, but it is more toxic than amphotericin B.

(2) It is used primarily in **topical preparations** but can be taken orally for **oral and esophageal candidiasis.** It is not for parenteral administration.

(3) It is not absorbed from skin, mucous membranes, or the gastrointestinal tract.

b. **Indications for use** include **candidal infections** of skin, mucous membranes, and vagina; and prophylaxis to prevent intestinal fungal overgrowth in patients on chemotherapy.

B. **Imidazoles.** These agents inhibit 14-alpha-demethylase and **block the synthesis of fungal cell membrane ergosterol,** leading to increased membrane permeability and loss of essential nutrients.

1. **Miconazole and clotrimazole**

a. **Pharmacologic properties.** Miconazole and clotrimazole are **topically active antifungals.**

b. **Indications for use.** The drugs are used in the treatment of **ringworm** and **vulvovaginal candidiasis.** Intravenous miconazole is rarely used because of toxicity.

2. **Ketoconazole, itraconazole, and fluconazole**

a. **Pharmacologic properties**

(1) Administered orally

b. **Indications for use**

(1) Ketoconazole is used to treat coccidioidomycosis, histoplasmosis, blastomycosis, paracoccidioidomycosis, and mucocutaneous candidiasis.

(2) Itraconazole is used in blastomycosis, histoplasmosis, and aspergillosis.

(3) Fluconazole is used in treatment of cryptococcal meningitis in AIDS patients and in severe cases of candidiasis.

NOTE

Aspergillus are very resistant to these imidazole antifungals. Aspergillosis is treated with amphotericin B.

Basic Immunology

The immune system is an intricate collection of organs, tissues, cells, and soluble factors that allow individuals to defend against harmful agents such as viruses, bacteria, and tumor cells. The immune system includes the primary or central lymphoid organs in which the leukocytes develop, the secondary or peripheral lymphoid organs and tissues in which immune responses occur, and the leukocytes circulating in the blood. The first two sections will review the cells and organs that comprise the immune system. The third section will present the basic characteristics of immune responses, including antigens, antibodies and T-cell receptors, and mechanisms of regulation. The fourth section will examine T-cell and B-cell activation, the cellular interactions necessary for cell-mediated (e.g., T-cell) and humoral (e.g., antibodies) responses and the cytokines that influence these processes. The fifth section will discuss complement and inflammation, while the last section will examine important immunological laboratory methods.

CELLS OF THE IMMUNE SYSTEM

Leukocytes include lymphocytes (B cells, T cells and large granular lymphocytes or NK cells), mononuclear phagocytes (monocytes and macrophages), polymorphonuclear granulocytes (neutrophils, eosinophils, and basophils), mast cells, and dendritic cells. All leukocytes, as well as erythrocytes and platelets, initially differentiate in the adult bone marrow (described below); most complete maturation there, although T cells finish their maturation in the thymus.

A. **Monocytes and macrophages** control infections that are not susceptible to neutrophil attack. They are derived as follows: stem cell → monoblast → promonocyte → circulating monocyte → tissue macrophage. Macrophages have a long life span compared to neutrophils.

NOTE

Cells of the immune system mature in the bone marrow (B cells and phagocytic cells) or in the thymus (T cells).

1. **Function of monocytes and macrophages.** These cells play a central role in cell-mediated immunity. They ingest particles via pinocytosis or phagocytosis and modulate inflammation via secretion of mediators. They also act as cells that process and present antigens to T lymphocytes (antigen-presenting cells).
   a. **Macrophages secrete over 100 mediators,** including interleukins 1, 8, and 12, collagenase, elastase, lipase, proteases, prostaglandins, leukotrienes, thromboxanes, lysozyme, and interferon. They also secrete complement components.
   b. **Macrophages have Fc receptors and class II MHC molecules** on the cell surface that mediate their biological functions. The Fc receptors allow the uptake of immune complexes (IgG complexed to antigen) and class II molecules present antigenic peptides to T cells.
   c. **Circulating monocytes differentiate into tissue macrophages** with specific names. For example, tissue macrophages present within the sinusoids of the liver are called **Kupffer cells.** Macrophages found in the lung are termed **alveolar macrophages;** in the brain, **microglial cells.**
      (1) Kupffer cells encounter antigens first from intestinal lumen absorption and serve to clear particulate and soluble matter from portal circulation. They phagocytose bacterial endotoxin, soluble immune complexes, activated clotting factors, and microorganisms.
      (2) Alveolar macrophages destroy inhaled antigens and microbes.
2. **Morphology of macrophages**
   a. **Epithelioid cells,** usually found in granulomas, are derived from blood monocytes and are activated by an immune response to antigen.
   b. **Multinucleated giant cells** are formed by the fusion of macrophages.
3. **Macrophage activation.** Macrophages are stimulated by lymphokines to kill microorganisms and tumor cells.
   a. Activated macrophages have increased lysosomal hydrolytic enzymes and an increased chemotactic response. C5a, and various cytokines from lymphocytes, neutrophils, and fibroblasts are chemoattractants for activated macrophages.
   b. Antigen coated with appropriate complement proteins (e.g., C3b) or antibody is more readily phagocytosed. This process is called **opsonization.**

NOTE

The Fc receptors on macrophages are essential for opsonization of bacteria by antibody. They react with the Fc region of IgG antibody molecules and hold the microbe close to the phagocytic cell membrane, thus facilitating the engulfment process.

c. Morphologic changes occur during activation, and include increases in size, number of pseudopods, and pinocytotic vesicles.

d. Macrophages experience a respiratory burst during phagocytosis via the **hexose-monophosphate shunt** pathway. This is a source of energy needed for cell membrane synthesis and also generates toxic oxygen metabolites such as singlet oxygen, superoxide anion, and hydrogen peroxide.

4. **Macrophages present antigens to T lymphocytes.** Antigen undergoes phagocytosis or pinocytosis. Once in the cytoplasm of the macrophage, the antigen is degraded into small peptides. The peptides are then noncovalently bound to class II MHC molecules in an endosomal vesicle and the complex is then transported to the cell surface, where it can now stimulate class II-restricted antigen-specific helper T cells.

IN A NUTSHELL

Macrophages process exogenous antigens and present the epitopes in a groove of the class II MHC molecules. T cells bearing receptors specific for the epitope will react with the epitope/MHC complex and be triggered to release lymphokines.

B. **Dendritic cells** are present in peripheral blood and lymphoid organs. Their primary function is to digest and process antigen for presentation to T cells. Dendritic cells include Langerhans' cells of skin, veiled cells in afferent lymphatics, and interdigitating reticulum cells in spleen and lymph nodes.

C. **Granulocytes or polymorphonuclear leukocytes.** There are three types of granulocytes: neutrophils, eosinophils, and basophils.

1. **Neutrophils (polymorphonuclear leukocytes, PMNs)** represent 60% of circulating blood leukocytes. 75-90% of circulating neutrophils have a receptor for the Fc region of IgG (FcγR).

a. Neutrophils have a multilobulated nucleus with dense chromatin and cytoplasmic lysosomes containing peroxidases and acid hydrolases.

b. They reach the tissues by diapedesis, inserting pseudopodia between the endothelial cells and dissolving the basement membrane, so that movement through blood vessel wall occurs.

c. Neutrophils are the first cells to arrive at acute inflammatory sites. They actively kill bacteria; their half-life is 6-20 hours.

d. Cytoplasmic granules contain digestive enzymes. These include azurophilic granules that contain myeloperoxidase and specific granules that contain lactoferrin.

e. Neutrophils phagocytize and then kill the organism by generation of H_2O_2 and toxic oxygen radicals and via the action granule-derived enzymes.

IN A NUTSHELL

PMNs kill microbes via:
- *Toxic oxygen metabolites*
- *Digestive enzymes present in lysosomal granules*

CLINICAL CORRELATE

Eosinophilia is a hallmark of :
- *Atopic allergies*
- *Worm infestations*

It can also be seen in collagen vascular diseases and neoplastic disorders.

NOTE

Eosinophil granule contents that help control allergic reactions include histaminase and aryl sulfatase.

2. Eosinophils represent 1-3% of circulating leukocytes.
   a. Approximately 50% of circulating eosinophils have receptors for complement.
   b. Eosinophils have a bilobed nucleus and contain crystalloid granules staining red with Giemsa.
   c. Eosinophil chemotactic factors include histamine, C5a, HETE, immune complexes, and ECF-A (eosinophil chemotactic factor of anaphylaxis).
   d. They are functionally important in late inflammatory reactions, particularly in **parasitic infections** and **allergy.**
   e. Some important contents of eosinophils and their functions include:
      (1) **Histaminase** degrades histamine.
      (2) **Pyrogen** produces fever.
      (3) **Peroxidase** kills microorganisms.
      (4) **Aryl sulfatase** degrades leukotrienens C_4, D_4, and E_4.
      (5) **Major basic proteins** are toxic to worms.
3. **Basophils** represent 1% of circulating leukocytes and are the smallest type of granulocytes.
   a. They contain abundant granules with RNA, mucopolysaccharides, and hypersensitivity mediators.
   b. They have receptors for the Fc portion of IgE.
   c. IgE binding promotes degranulation as it does in the tissue mast cells. Histamine and other mediators are released during degranulation and are responsible for the symptoms seen in atopic allergies. This is discussed further in the Clinical Immunology chapter.

D. **Lymphocytes,** which include B and T lymphocytes, represent **30%** of circulating leukocytes. Lymphocytes have a high nucleus-to-cytoplasm ratio and are distinguished by their antigen receptors and cell surface markers.

1. **B lymphocytes.** B lymphocytes differentiate into **plasma cells,** which secrete large amounts of immunoglobulin.
   a. There are two major subsets of B lymphocytes (from mouse models). **Lyb 5-positive cells** produce antibody to soluble polysaccharides. They are stimulated by nonspecific lymphokines from T-helper cells. **Lyb 5-negative cells** produce antibody to protein antigens, cellular antigens, and bacterial lipopolysaccharides. These cells require direct physical interaction with specific helper T cells.
   b. **Memory B cells,** generated after the primary exposure to an antigen, secrete antibody with increased affinity for the antigen.

c. **Mature B cells** have surface IgM and IgD that bind antigen, causing the B cell to become active and secrete immunoglobulins.

d. B cells respond to two types of antigens.

(1) **T-cell-independent antigens** stimulate B cells to secrete immunoglobulin in the absence of $CD4^+$ helper T cells.

(2) **T-cell-dependent antigens** (most all protein antigens) regulate B and T cell interaction before immunoglobulin is produced. The T cell then drives B cells to secrete antibody via direct contact and by the lymphokine secretion of T cells.

2. **T lymphocytes.** Two major types of T cells exist, and are classified based on the expression of the cell surface proteins CD4 or CD8. Cellular differentiation (CD) proteins are present on many cells of the body. They reflect the function of the cell and are thus "markers" for particular cells. For example, CD3 is found in the membrane of all mature T cells, where CD4 and CD8 proteins are found on certain subsets of these mature T cells.

a. **Helper T cells are CD4 positive.** There are two distinct subsets of helper T cells with different functions. One function is to stimulate B lymphocytes to proliferate and differentiate into antibody-producing cells. A second function of $CD4^+$ T cells is to promote cytotoxic T-cell ($CD8^+$ cells) responses. Cellular differentiation (CD) proteins are present on many cells of the body.

(1) Activation and proliferation of helper T cells depends on corecognition of specific antigenic peptides and MHC class II molecules on antigen-presenting cells such as macrophages.

(2) Activated helper T cells produce **lymphokines** (including B-cell growth factor), differentiation factors, and inflammatory ctyokines.

b. **Cytotoxic T cells are also CD8 positive.** They lyse cells carrying specific antigens such as virus infected cells and tumor cells. Cytotoxic T cells act by recognizing foreign antigen and MHC class I molecules with their T-cell receptor.

c. **Cells with suppressor function are CD8 positive.** These cells suppress immune responses via the soluble lymphokines they release.

3. **Natural killer (NK) cells** represent 10-15% of lymphocytes in peripheral circulation.

a. NK cells kill certain tumor cells (without damaging normal tissues) and defend against viral infection. Unlike T cells,

NOTE

T-cell-independent antigens induce IgM antibody only and do not cause immunologic memory (anamnesis).

NOTE

There are two subsets of helper T cells. Th1 releases IL-2 and IFN-γ, while Th2 releases other interleukins (e.g., 4, 5, 6, 10). Th1 cells stimulate proliferation and cytoxic responses, while Th2 cells stimulate B-cell maturation, differentiation, and class switching.

IN A NUTSHELL

- *Helper T cells → $CD4^+$*
- *Cytotoxic T cells → $CD8^+$*

Another T-cell subset is the cell responsible for the delayed-type hypersensitivity (T_{DTH}) cell, which has CD4 in its membrane.

NK cells recognize foreign antigen in the **absence of MHC molecules.**

b. NK cells mediate **antibody-dependent cellular toxicity (ADCC).** This function allows NK cells to kill opsonized, or antibody coated, target cells.
c. NK cells are activated by cytokines such as gamma interferon.

LYMPHORETICULAR SYSTEM

The lymphoreticular system (Figure 1-1) is comprised of the primary lymphoid organs, in which hematopoiesis and lymphopoiesis occurs, and secondary lymphoid organs, in which immune responses occur. **Primary, or "central" lymphoid organs** in children and adults include the **bone marrow** and **thymus;** in the fetus, the liver is also a primary organ. Hematopoiesis occurs in the bone marrow, producing mature erythrocytes, platelets, monocytes, granulocytes and B cells, as well as precursors for T cells, NK cells, dendritic cells, and mast cells. The T cells finish maturation in the **thymus,** while the rest finish maturation in the periphery. The major **secondary** or **"peripheral" lymphoid organs** and tissues include the **lymph nodes, spleen,** and the **mucosa-associated lymphoid tissue system (MALTS).** MALTS includes the **gut-associated lymphoid tissue (GALT), bronchus-associated lymphoid tissue (BALT),** and submucosal lymphoid tissues of the genitourinary tract. The purpose of secondary lymphoid organs is to trap and present antigen to circulating lymphocytes in stimulating adaptive immune responses. These secondary tissues or organs protect all

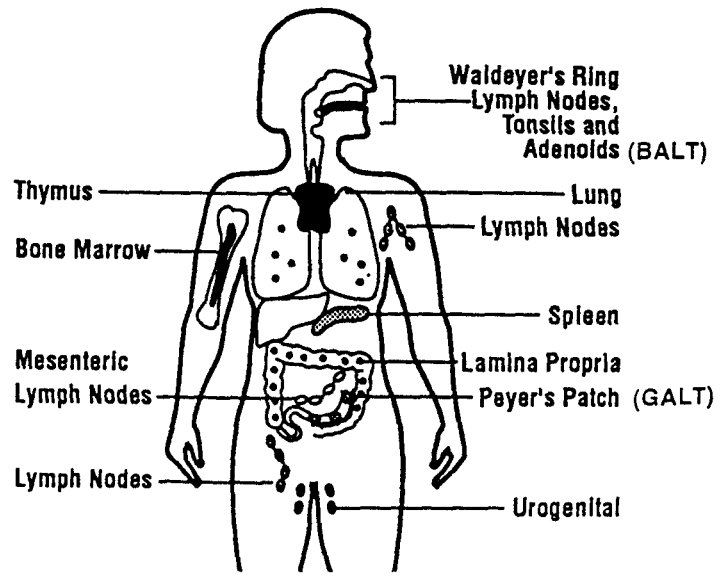


Figure 1-1. The lymphoreticular system.

surfaces and fluids of the body. The tissue fluid, or lymph, is filter

IN A NUTSHELL

Primary lymphoid organs:
- *Bone marrow*
- *Thymus*

Secondary lymphoid organs:
- *Lymph nodes*
- *Spleen*
- *Tonsils*
- *Mucosa-associated lymphoid tissue (MALT)*
  - *Gut-associated lymphoid tissue (GALT)*
  - *Bronchus-associated lymphoid tissue (BALT)*

through lymph nodes and the tissues of MALT while the primary filter for the blood is the spleen and to some extent the liver.

A. **Bone marrow structure and function.** Bone marrow is a primary organ because it is the site of hematopoiesis and B-cell maturation, as well as the site of origin of the stem cells involved in T-cell production. It can also be considered a secondary organ, since it is an important site for plasma cells to secrete antibody into the blood and it contains activated T cells as well.

1. **Bone marrow structure.** The bone marrow is a very large tissue comprising 3-5% of body mass in humans. It is found in the long bones of the body, in the cranium, and iliac crest. There are two functional components of approximately equal size: the vascular and adipose portion, and the hematopoietic portion. The latter is involved in the formation of blood cells, which are all derived from a single progenitor stem cell.
2. **Hematopoietic cell differentiation** (Figure 1-2). All blood cells are derived from pluripotent hematopoietic stem cells that differentiate into myeloid and lymphoid progenitor cells.

NOTE

Bone marrow is the source of pluripotent atem cells and the site of B-cell maturation.

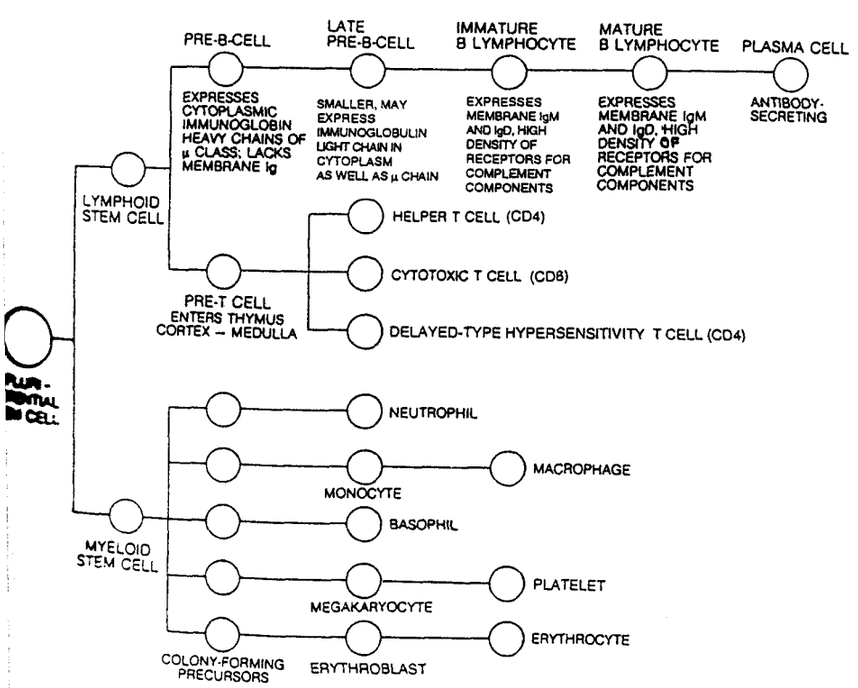


Figure 1-2. Hematopoietic cell differentiation.

a. Progenitor cells are acted upon by appropriate stimuli in certain anatomical sites, such as primary lymphoid organs (thymus and bone marrow) and secondary lymphoid organs (lymph nodes). Stimuli include colony-stimulating factors, erythropoietin, thymosin, and antigen (both self and foreign).
b. After maturation in the thymus or bone marrow, lymphocytes leave these sites and migrate to spleen, lymph nodes, and MALT (secondary lymphoid organs) where further development occurs under the influence of antigens and cytokines.

B. **Thymus structure and function.** Precursors of thymic (T) lymphocytes travel from the bone marrow to the thymus, where early differentiation and maturation take place, including T-cell receptor expression and clonal deletion of autoreactive T cells. T lymphocytes leave the thymus to seed the secondary lymphoid organs in thymic-dependent regions, where they can be activated and undergo their final maturation into effector T cells that function in immune responses.

1. **Development of the thymus.** The thymus is derived from the third and fourth pharyngeal pouches and is found in the mediastinum.
   a. The development of the thymus begins with epithelial (endodermal) outgrowths of the third and fourth pharyngeal pouches.
   b. Subsequently, this epithelial reticulum is infiltrated by lymphocytes and other mesodermal elements.
   c. The thymus reaches its maximum weight at puberty and then, slowly involutes as it becomes infiltrated with connective and adipose tissues.
   d. There is a significant amount of programmed cell death (apoptosis) that occurs in the thymus. This is a reflection of the elimination of autoreactive lymphocytes during differentiation in this organ.
2. **Organization of the thymus.** The **stroma** of the thymus consists of a prominent connective tissue capsule, which invaginates into the parenchyma as septa and divides the thymus into lobules. The parenchyma is organized into a cortex and medulla.
   a. **Cortex** is composed of tightly packed differentiating lymphocytes surrounded by a meshwork of epithelial reticular cells and macrophages.
   b. **Medulla** consists of epithelial reticular cells and lymphocytes. The medulla exhibits a paler staining than the cortex as a result of the large reticular cells and the presence

larger lymphocytes that are not as densely packed. The medulla also contains the characteristic **Hassal's corpuscles,** which are composed of concentrically arranged dead and dying reticular cells, macrophages, neutrophils, and nuclear material whose origin is unknown.

3. **Blood supply.** Arteries from the connective tissue capsule and septa enter the thymus at the level of the corticomedullary junction. These arteries branch to give off capillaries, which loop up to the periphery and turn back to the medulla to form venules, which leave the septa.
   a. **Thymic capillaries** have a nonporous endothelium with a thick basement membrane.
   b. **Epithelial reticular cells** surround thymic vessels and constitute an incomplete barrier, which separates the blood from the lymphocytes. The thymic reticulum is composed of branching epithelial cells that are joined one to another by desmosomes.
4. **Thymus** is large at birth in relation to other organs; it increases in size until puberty, when involution begins. **Thymectomy** in young animals results in poor development of the other lymphoid tissues and the absence of cell-mediated immunity. This can be reversed by a thymic graft. Congenital absence of the thymus (i.e., **DiGeorge's syndrome**) results in poor development of peripheral lymphoid tissues and the absence of cell-mediated immunity.
5. **Thymosin,** a lymphokine that stimulates thymus-dependent zones in the peripheral lymphoid tissues, is a hormone produced by the thymus.

C. **Lymphatics.** More plasma is filtered from capillary beds into the tissues of the body than is reabsorbed back into the venous end of those capillary beds. This excess fluid moves through the tissues or organs as **interstitial fluid,** then is collected in small lymphatic vessels throughout the tissues as **lymph.** These small lymphatics fuse into larger afferent lymphatics that enter lymph nodes. The efferent lymphatic leaving one node may become the afferent lymphatic entering another node in a cluster. Eventually, the final efferent lymphatics fuse into the large **thoracic duct** that extends the length of the thorax and empties into the left subclavian vein, returning the lymph fluid to the blood.

D. **Lymph nodes** are highly organized secondary organs that are the most common site for an adaptive immune response, since they filter the lymph that washes through the body tissues. During an infection, lymph nodes may increase two to five times in size. They are encapsulated, kidney-shaped structures that have a concave side with a hilum. The blood vessels and nerves enter and

IN A NUTSHELL

Thymus cortex:
- *Darker peripheral zone*
- *Extensive population of T cells, epithelial reticular cells, and macrophages*

Thymus medulla:
- *Lighter central zone*
- *Larger number of epithelial reticular cells, and large- and medium-sized lymphocytes*
- *Hassal's corpuscles*

CLINICAL CORRELATE

DiGeorge's syndrome is the absence or atrophy of the thymus, resulting in reduced level of B cells and severe decrease in T cells. Afflicted individuals have compromised B-cell immunity and no T-cell immunity. DiGeorge's syndrome is often accompanied by hypoplasia of the parathyroid glands with resultant tetany. Early death from severe infection often occurs.

exit at the hilum, and the efferent lymphatic exits from this region. Beneath the stromal capsule, the parenchyma is organized into a cortex and medulla.

1. The **stroma** consists of a dense connective tissue capsule that surrounds each lymph node and sends collagenous trabeculae into the node to divide its parenchyma into incomplete compartments (Figure 1-3).
   a. Reticular cells produce reticular fibers that anastomose with the trabeculae and form an extensive network.
   b. The delicate reticulum filters the lymph and suspends the lymphocytes and macrophages.
   c. This stromal organization and function facilitates cell-to-cell and antigen-receptor interactions.
2. **Cortex.** Beneath the capsule (except at the hilum) lies a cortex, which is composed of lymphatic nodules in a diffuse lymphatic tissue network that intermingles with the subcapsular and peritrabecular sinuses.
   a. **Lymphatic sinuses** are the lymphatic passageways within lymph nodes. They are lined with flat endothelial cells in the area of the capsule and are partially lined by reticular cells and fibers elsewhere. The sinuses receive lymph brought by afferent vessels and transport it toward the medulla.

CLINICAL CORRELATE

Each node receives lymph from a defined and limited region of the body. Neoplasms can metastasize via these nodes. For example, a common metastatic site for breast cancer is the axillary node under the arm.

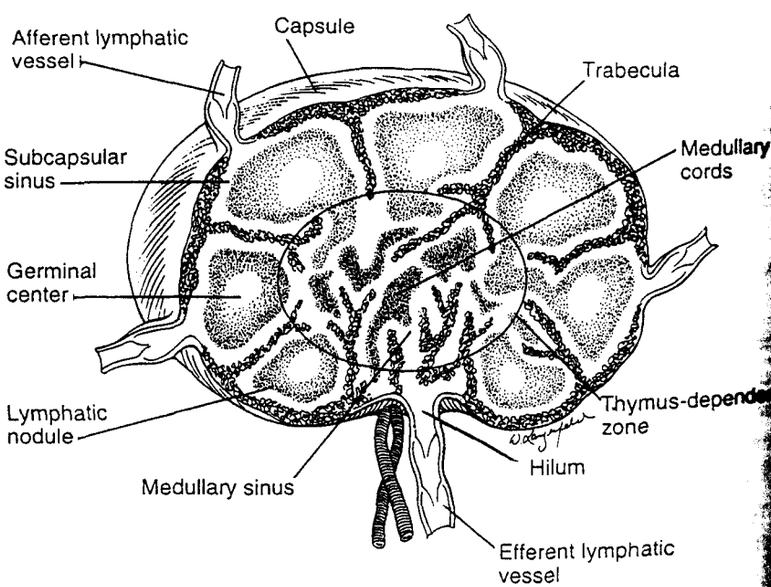


Figure 1-3. Lymph node.

b. **Germinal centers** may be present in the nodules of the cortex (see Figure 1-3). They are composed of lymphocytes and macrophages and are transient structures in which new antigens are localized and degraded. Antigen stimulation increases the number and development of germinal centers. Germinal centers are clones of B cells developing into plasma cells in response to a specific antigen.

3. The **medulla** of the lymph node occupies the center of the organ. It contains medullary cords composed of lymphoid tissue that extend from the cortex. **Medullary sinuses,** like those in the cortex, transmit the lymph toward the hilum, where it exits through the efferent lymphatic.

4. **Function.** Lymph nodes serve as filters clearing lymph of foreign particles before circulating to other areas of the body.
   a. Lymph enters the node via afferent lymphatic vessels, which are located on the convex side of the organ (see Figure 1-3).
   b. From the afferent lymphatics, lymph passes through the subcapsular sinus to the peritrabecular sinuses. It then enters the medullary sinuses and exits the lymph node via the efferent lymphatic vessels at the hilum.

E. The **spleen** is a peripheral lymphoid organ in the upper left quadrant of the abdominal cavity, which acts as a storage depot for blood, clears old and defective erythrocytes (RBCs), and provides protection from blood-borne pathogens.

1. The **stroma** of the spleen consists of the following:
   a. A dense connective tissue capsule that contains smooth muscle cells.
   b. Trabeculae that branch off of the capsule and partially partition the parenchyma of splénic pulp.
   c. A delicate meshwork of reticular connective tissue that filters the blood.

2. Splenic **parenchyma** consists of **white and red pulp.**
   a. **White pulp** consists of lymphatic tissue arranged in sheaths around arterioles and in nodules. Antigenic stimulation increases the amount of white pulp.
      (1) The **periarteriolar lymphocyte sheaths (PALS)** are accumulations of diffuse lymphatic tissues that are rich in T cells. The marginal zone is occupied mostly by B cells.
      (2) B lymphocytes cluster peripherally in the PALS to form primary follicles. After antigenic stimulation, these follicles develop into secondary follicles with germinal centers containing rapidly dividing B cells.

CLINICAL CORRELATE

Patients with sickle cell disease have a functionally-impaired spleen. This predisposes them to septicemia, particularly those caused by Streptococcus pneumoniae. Other opportunists include Salmonella, Meningococci, and H. influenzae.

NOTE

In the splenic white pulp, B and T lymphocytes are segregated—the central portion of PALs are rich in T cells, while the marginal zone and nodules are populated by B cells.

(3) At the **marginal zone,** dendritic cells trap and proces antigen and migrate to PALS to present it to antigen specific cells of the immune system.

b. **Red pulp** consists primarily of erythrocyte-filled sinusoid and macrophages in a reticular fiber network; most filtra tion occurs here.

(1) The sinuses vary in size and are separated by pulp (i.e. Billroth) cords.

(2) RBCs and platelets are exposed to macrophages in th pulp cords; macrophages phagocytize worn-out or dam aged cells.

(3) Splenic sinusoids are lined by loosely arranged endothe lial cells, which have numerous fenestrations. Thes sinusoids are surrounded by a poorly developed basa lamina.

(4) Phagocytosis is carried out primarily by macrophages located outside the sinusoids. The macrophages extend finger-like projections into the sinusoids, which push through the discontinuous basement membran between the endothelial cells.

3. **Blood supply.** Arteries from the hilum of the spleen pass along trabeculae to enter the periarteriolar sheath.

a. These vessels branch like brush bristles to form **penicillar arteries,** which continue in capillaries and open blindly in the red pulp or connect directly to venous sinusoids.

b. The **venous sinusoids** are specialized, large caliber vessels which are bordered by elongated endothelial cells and held in place by circularly arranged reticular fibers.

c. Venous sinusoids drain into veins that eventually exit at th hilum.

F. **Gut-associated lymphoid tissue (GALT)** is not encapsulated and present on mucosal surfaces in the gastrointestinal tracts. It is th site of immune responses to injested microbes and some food antigens.

1. **Structure.** Lymphoid tissue in GALT includes the large follicu aggregates in the small intestine called **Peyer's patches,** **lamina propria** beneath the mucosal epithelia in the villi, a the **intraepithelial lymphocytes (IELs)** found between muco epithelial cells along the surface of the villi.

2. **Function**

a. The lymphoid tissues lining the intestinal tract are exemplified by the Peyer's patches. Structurally unl antigen-presenting cells, called **M cells,** are located mucous membranes. They endocytose microbes and

gens and present specific epitopes to B lymphocytes clustered in a deep invagination, or pocket, in the basolateral cytoplasmic membrane. The B cells become activated and migrate to MALT where they differentiate into IgA-secreting plasma cells.

b. The IgA dimers react with a poly-immunoglobulin receptor on intestinal epithelial cells. The dimer (held together by the J chain) is internalized and crosses the epithelial cell cytosol. It is then proteolytically cleaved from the poly-Ig receptor and excreted into the lumen of the intestine. A small portion of the Ig receptor remains attached to the dimer; this is called the **secretory component.** The function of this small peptide is to protect the antibody molecule from enzymatic hydrolysis in intestinal fluid.

Bronchus-associated lymphoid tissue (BALT) includes the lymphoid tissue beneath the respiratory mucosa and the aggregates of nodular lymphatic tissue called **tonsils**. These are structurally similar to lymph nodes and contain deep crypts that allow antigen to be trapped, degraded, and processed by antigen-presenting cells, such as macrophages and dendritic cells.

1. **Organization of the tonsils.** Tonsils are peripheral lymphoid organs composed of aggregates of nodular and diffuse lymphatic tissues that protect epithelial surfaces. The lymphoid tissues are located beneath the epithelium in the underlying connective tissues.
   a. Lymphatic nodules contain clones of B lymphocytes, which differentiate into plasma cells to produce antibodies for the humoral immune response.
   b. T cells are found primarily in diffuse lymphatic tissues.
2. **Types of tonsils.** Three important examples of tonsillar tissue are the **palatine tonsils,** the **lingual tonsils,** and the **pharyngeal tonsil.**
   a. **Palatine tonsils** are located bilaterally in the oropharynx.
      (1) They are composed of dense lymphoid tissue, which forms a band of lymphatic nodules with germinal centers. These are intermingled with diffuse lymphatic tissue beneath the stratified squamous epithelium.
      (2) A dense connective tissue capsule often separates the tonsil from subjacent tissues.
      (3) Each tonsil has numerous epithelial invaginations, or **crypts,** which contain desquamated epithelial cells, lymphocytes, and bacteria in their lumina.
   b. **Lingual tonsils** are smaller and more numerous aggregates of lymphoid tissues located at the base of the tongue.

CLINICAL CORRELATE

Peyer's patches and other lymphoid tissues will be hypoplastic in individuals with Bruton's hypogammaglobulinemia. Lymphoid tissue is almost totally absent in patients with severe combined immunodeficiency.

(1) They are covered by the stratified squamous epitheli
on the dorsum of the tongue.
(2) Each aggregate possesses a single crypt.

c. **Pharyngeal tonsil** is an unpaired accumulation of lympho
tissue located on the posterior wall of the nasopharynx.
(1) It is usually covered by a pseudostratified column
epithelium with cilia and goblet cells; however, t
epithelium can be obscured by an infiltration of ly
phocytes or by metaplasia (seen in smokers). The p
ryngeal tonsils, or hypertrophic pharngeal tonsils,
often referred to as **adenoids.**
(2) Instead of forming crypts, the overlying epitheli
occurs in a series of folds.

H. **Lymphocyte recirculation**

1. Lymphocytes are capable of high levels of recirculation, co
tinuously moving from the blood out into the tissues and th
returning via the lymphatics.
2. As these cells circulate, they make contact with epitopes pr
sented in the groove of class II MHC molecules located on th
surface of antigen-presenting cells. This dynamic greatl
enhances the efficiency of B- and T-cell triggering by increa
ing the frequency of specific interactions.
3. Once the triggering has occurred, the committed lymphocyt
rapidly "home" to peripheral lymphoid tissues. It has be
demonstrated that T cells specific for a particular epitope w
disappear from circulation within 48 hours after antigen ex
sure. This homing is accomplished by the interaction of cell
cell adhesion molecules.
4. The lymphocytes bear in their membranes **selectins** that in
act specifically with **addressins** that are found in the lymph
vasculature, particularly in the postcapillary venules. It is
that specialized cells with a plump cuboidal shape are fo
these are called high endothelial venules due to the heig
the region caused by the cuboidal cells.
5. Different populations of lymphocytes express diffe
homing receptors; B cells express an integrin that r
with vascular addressins in MALT, whereas T cells ex
a selectin for addressins in peripheral lymphoid ti
(hence T cells predominate in lymph nodes and B ce
Peyer's patches).

NOTE

Lymphocyte homing is controlled by selectins in the lymphocyte membrane that react specifically with addressins found in the lymphatic vasculature.

IE IMMUNE RESPONSE

mune cells and the responses they generate can be categorized :o innate and acquired. The innate responses are necessary for mediate reponse to a pathogen and for establishing a local 'lammation to recruit circulating phagocytes and, later, activated cells and B cells. The T and B lymphocytes are the acquired cells id are responsible for many of the classic characteristics of the mune response, including memory, antigen specificity, and toler- ice to self. The characteristics of antigen presentation and recogni- on by T cells and B cells will be discussed below.

Natural versus acquired immunity. The immune system is composed of cells that defend against foreign invaders by both nonspecific mechanisms (termed natural or innate immunity), and antigen-specific mechanisms (known as acquired immunity). The nonspecific response is typically observed first since no prior exposure to antigen is necessary. This response may not be sufficient to clear the foreign antigen and an antigen-specific mechanism directed by B and T cells may be required.

1. **Natural immunity** (also known as innate immunity) is present at birth in all individuals. This response does not increase upon repeated exposure to a given antigen.
   a. The first line of defense is intact skin and mucous membranes.
   b. Natural immunity allows elimination of foreign substance without previous exposure.
   c. This immunity is effected by either natural antibodies or natural cytotoxic cells, including macrophages, neutrophils, eosinophils, and natural killer cells.
2. **Acquired immunity** is established late in fetal life and development continues during childhood. Continued exposure to foreign antigen will stimulate acquired immunity.
   a. Responses are specific to individual antigens because of antigen specific recognition by surface antibody on B cells and by T-cell receptors (TCRs) on T cells.
   b. Individual lymphocytes recognize their appropriate antigens (discussed below) and are activated to proliferate into a **clone of effector cells and memory cells** with the same antigen specificity (clonal expansion).
   c. May have **anamnestic response** in which subsequent response to previously recognized antigen is more specific and often magnified (this is a result of the production of memory T and B cells).
   d. Antigen is eliminated due to specific antigen recognition and effector functions (discussed below).

IN A NUTSHELL

Natural immunity:
- *Present at birth*
- *Comprised of skin, mucous membranes, secretions such as saliva and tears, phagocytic cells, and NK cells*

Acquired immunity:
- *Developed in response to immunogen exposure*
- *Comprised of antibodies (IgG, IgA, etc.) and sensitized lymphocytes (B and T cells)*

NOTE

*Thymus-independent antigens do **not** induce memory cell production.*

3. **Self-tolerance** describes the absence of immune responses to one's own tissue antigens. It is necessary to prevent autoimmune responses. Mechanisms of self-tolerance include the following:
   a. **Clonal deletion**, the elimination of clones of developing T-cells with receptors against self-antigens, occurs during cell maturation in the thymus.
   b. **Suppression of peripheral T and B lymphocytes** bearing receptors for self-antigens is maintained by the lack of costimulatory molecules on the surface of the naive cells.

B. **Antigens and antibodies.** An **antigen** is any substance that can be specifically bound by an antibody or T-cell receptor, while an **immunogen** is that which induces an immune response. Since antibodies and TCRs recognize different types of antigens, and antibody production requires helper T-cell activation and assistance, immunogens typically possess both antibody and T-cell reactive regions.
   1. An **epitope** or **antigenic determinant** is a specific site on the antigen that is recognized by the immune system, i.e., the part of the antigen to which an antibody binds.
      a. Most antigens express two or more epitopes.
      b. B-cell epitopes usually occur at the hydrophilic surface, while T-cell epitopes may be embedded within the protein.
   2. **Antibodies** are able to bind to epitopes on a wide variety of molecules, including proteins, carbohydrates, nucleic acids and small organic molecules. **T-cell receptors (TCRs)** are only able to recognize peptides bound to **major histocompatiblity (MHC)** proteins on the surface of a cell (discussed below).
   3. Many macromolecules can be antigenic.
      a. **Proteins** (glycoproteins, lipoproteins, or nucleoproteins) are the most common form of antigen and are usually very good immunogens.
         (1) Their antigenicity is based on amino acid composition, three-dimensional conformation, and/or biochemical properties (such as charge, etc.).
         (2) Peptides derived from processed proteins and bound to cell-surface MHC proteins are the only type of antigen that T-cell receptors can recognize.
      b. Large, repetitive **polysaccharides** can activate B cells with little or no helper T aid (but cannot be recognized alone by TCRs) and so are considered T-cell-independent antigens.

c. **Nucleic acids** can be recognized by antibodies, but are poor immunogens because they cannot act as T-cell antigens unless they are bound to a specific protein carrier.
d. **Lipids** are usually not immunogenic. When lipids are coupled to protein antigens, they tend to induce T-cell mediated delayed hypersenstivity rather than antibody production.
e. **Haptens** are small molecules that can act as an antibody epitope, but will not induce immune responses since they are not recognized as T-cell antigens. When combined with a carrier protein, the hapten-carrier complex can produce hapten-specific and carrier-specific antibodies, since the helper T cell can recognize carrier peptides. Many allergens (e.g., penicillin) are haptens.

C. **Major histocompatibility complex (MHC).** The MHC is a collection of highly polymorphic genes encoding the proteins that regulate immune responses. These genes include, most notably, the **class I** and **class II** cell surface proteins, and the **class III genes** that encode complement proteins. In humans, the MHC genes are termed **HLA (human leukocyte antigens)** and are found on the short arm of chromosome 6. The HLA proteins are glycoproteins present on cell surfaces that enable T cells to recognize and bind antigenic peptides, i.e., they function in immune recognition.

1. **HLA class I antigen**. Class I proteins are membrane glycoproteins on the surface of all nucleated cells and platelets. They bind peptides processed from protein synthesized in the cell's cytosol, and are necessary for antigen recognition by $CD8^+$ cytotoxic T lymphocytes (CTLs). In humans, the three types of class I genes are referred to as **HLA-A, HLA-B,** and **HLA-C** antigens. **Cytotoxic T cells** recognize viral, intracellular bacterial, parasitic, or tumor antigens in association with class I molecules. CD8 on the surface of these cells recognize a nonpolymorphic region of the class I MHC molecule.
2. **HLA class II antigen**. HLA class II proteins are expressed on a more restricted set of cells, including antigen-presenting cells (dendritic cells, Langerhans cells, activated macrophages), B cells, and thymic epithelial cells involved in T-cell maturation. These proteins bind peptide epitopes processed from endocytosed molecules and are necessary for **antigen recognition by helper T cells (T_H)** and **T cells that mediate delayed-type hypersensitivity (T_{DTH}).** Class II genes encode for cell surface glycoproteins with two polypeptide components called α and β (Figure 1-5). In humans, the class II genes include **HLA DR, DQ,** and **DP. Helper T and T_{DTH} cells** recognize viral, bacterial, parasite, or injected proteins in association with class II.

NOTE

Carrier effect:
Poorly immunogenic or nonimmunogenic molecules acquire immunogenicity when they are chemically linked to proteins that serve as carriers and impart the diversity and T-cell reactivity needed.

IN A NUTSHELL

Class I MHC:
- *All nucleated cells*
- *Human class I genes = HLA-A, HLA-B, and HLA-C*
- *Cytotoxic T cells (CD8) recognize class I MHC on infected cells (viruses, intracellular bacteria, parasites, tumor antigens)*

Class II MHC:
- *Limited to cells that interact with helper T cells = antigen-presenting cells (B cells, monocytes, macrophages, Langerhans cells, dendritic cells, thymic epithelium)*
- *Human class II genes = HLA-DR, HLA-DQ, and HLA-DP*
- *Helper T cells (CD4) bind to class II MHC on antigen-presenting cells*

NOTE

MHC class III proteins have nothing to do with transplant acceptance or rejection. They are proteins involved in innate immunity.

3. **HLA class III antigen.** Class III genes encode for complement components or regulators of serum complement component levels. C2 and Factor B are encoded by class III MHC genes.
4. **HLA disease associations.** Many diseases are associated with increased frequency of certain HLA antigens. Ankylosing spondylitis is typically associated with HLA-B27; other diseases are also associated with specific HLA antigens: rheumatoid arthritis (DR4), Sjögren's syndrome (DR3), and insulin-dependent diabetes mellitus (DR3 and DR4).
   a. Most of the HLA-associated diseases are of unknown etiology and have associated immunologic abnormalities.
   b. Tissue typing for organ transplantation involves matching the HLA class I and class II antigens.

D. **Antibodies.** Antibodies act as antigen-specific receptors on B cells, and when secreted by plasma cells, mediate humoral responses. Antibodies comprise approximately 20% of plasma proteins. They are produced by B cells in response to the introduction of foreign substances (i.e., antigens) into the body. Antibodies are bifunctional: they bind epitopes on antigens, thereby directly attacking the antigen, and they stimulate other biologic phenomena such as activating complement and binding Fc receptors on other lymphoid cells.

1. **Structure.** Antibodies consist of four polypeptide chains—**two identical light chains** and **two identical heavy chains**—all bound together by disulfide bonds (see Figure 1-4). Heavy and light chains have **hypervariable regions** at the amino terminus (responsible for antigen-binding specificity) and **constant regions** at the carboxy terminus. There are intrachain disulfide links that divide each chain into subunits of 110 amino acids; thus light chains have 2 domains referred to as the variable and constant domains, whereas heavy chains have 4 or 5 domains—1 variable domain and 3 or 4 constant ones.
   a. **Light chains.** The molecular weight of each light chain is approximately 23 kDa and is composed of 220 amino acids. There are two subtypes of light chains, kappa and lambda.
      (1) **Kappa chains** differ from **lambda chains** on the basis of structural differences in the constant region. The genes coding for each are not on the same chromosome.
      (2) Each antibody molecule has either two kappa or two lambda chains.
      (3) A specific immunoglobulin always has identical kappa or lambda chains.
      (4) The ratio of chain subtypes is constant within a species.

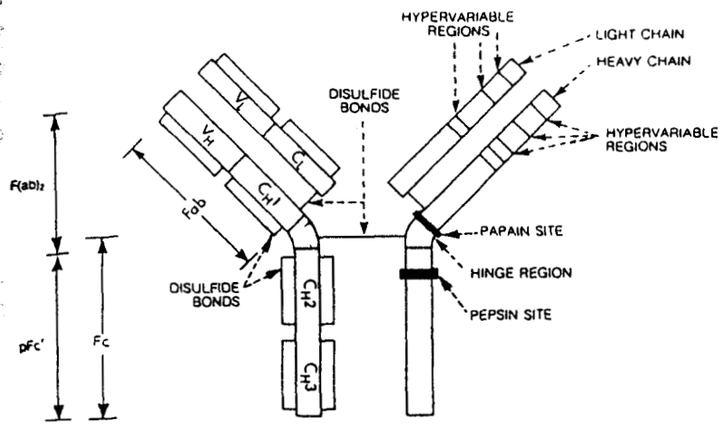


Figure 1-4. Schematic structure of immunoglobulin molecule.

b. **Heavy chains.** The molecular weight of each heavy chain is 50-75 kDa and is composed of 450-580 amino acids.
   (1) There are **five different isotypes** corresponding to the individual heavy chain gene utilized. Structural differences in the constant regions of the various types of heavy chains account for their different biological properties.
      (a) IgG has gamma (γ) heavy chains.
      (b) IgA has alpha (α) heavy chains.
      (c) IgM has mu (μ) heavy chains.
      (d) IgE has epsilon (ε) heavy chains.
      (e) IgD has delta (δ) heavy chains.
   (2) Heavy chains are further divided into subclasses based on the number of interchain disulfide bridges. For example, IgG1 and IgG4 have 2 disulfide bonds, IgG2 has 4 disulfide bonds, and IgG3 has 15 disulfide bonds.
   (3) Genes coding for heavy chains are on the same chromosome.

2. **Antigen-binding sites** are found in the variable region located in the 110-amino acid segment of the amino terminal end of the heavy and light chains.
   a. The variability of amino acid sequence confers antigen-binding specificity to individual immunoglobulin molecules. This site has both framework and hypervariable or complimentarity determining subregions. **Framework subregions** are relatively conserved amino acid sequences that preserve the three-dimensional structure of the variable region and stabilize the hypervariable regions. **Hypervariable regions** are folded to form the antigen-binding site (also called the paratope).

IN A NUTSHELL

Isotype	***Ig function***
IgG →	• *Opsonization* • *Placental passage* • *Complement activation*
IgA →	• *Mucosal (secretory) immunity*
IgM →	• *Complement activation*
IgE →	• *Basophil and mast cell sensitization*
IgD →	• *Antigen triggering of B cells*

b. Different structurally related antigens may be cross-reactive and bound by the same antigen-combining site on different antibodies. This is important in diseases such as rheumatic fever, where streptococcal antigens are structurally related and cross-reactive with heart tissue (cardiac myosin).

4. **Idiotypes, allotypes, and isotypes**
   a. **Idiotype** is the term used to describe the area of the variable region responsible for antigen specificity. These are the unique epitopes that are found in the paratope (see above) of the antibody.
   b. **Isotype** is the term used to describe the subclasses of immunoglobulins (G-A-M-E-D) that are distinguished by unique constant regions encoded by the heavy chain gene. The individual isotypes have unique effector mechanisms such as the ability to bind complement or mediate hypersensitivity responses (described below).
   c. **Allotype** is the protein product of an allele that may be detected as an antigen by another member of the same species. It involves different alleles at a specific site in the constant region of the heavy chain.
5. **Immune serum** contains a heterogeneous population of antibodies with varying affinity for antigens; the average affinity increases as the period of time after immunization lengthens due to somatic mutations.
6. **Monoclonal antibodies** are antibodies that are specific for a single epitope determinant. They are produced experimentally by **hybridomas,** created from the fusion of activated B lymphocytes to plasmacytoma cells that are grown in tissue culture.

E. **Properties of immunoglobulin subclasses**. As previously noted, there are five immunoglobulin subclasses: IgG, IgA, IgM, IgE, and IgD (remember them by "G-A-M-E-D"). They can be distinguished by the type of heavy chain they possess and by their distinct biological functions.

1. **IgG** provides the major defense against bacteria and toxins. There are four subclasses, varying by heavy chain isotype (G1, G2, G3, G4).
   a. IgG is important in the secondary immune response to an antigen and provides long-lasting immunity.
   b. IgG is the **only class of immunoglobulin that crosses the human placenta** into fetal circulation; it is responsible for protecting the newborn during the first 4-6 months of life.
   c. Three subclasses of IgG fix complement (IgG3 > IgG1 > IgG2).

NOTE

Monoclonal antibody:

B cell + plasmocytoma cell = hybridoma. The hybridoma secretes antibody identical to the one produced by the B cell. The plasmacytoma gives the hybridoma its immortality.

IN A NUTSHELL

Major functions of IgG:

- *Passive immunity to fetus*
- *Opsonization*
- *Complement activation*

2. **IgM** is important in the primary immune response to antigen, since it is usually the first antibody detected in the serum after exposure to a virus or specific antigen.
   a. Circulating IgM is a **pentamer** of five immunoglobulin molecules (10 heavy μ chains and 10 light chains) and one disulfide-linked J chain.
   b. IgM **fixes complement** very efficiently because each circulating molecule has ten Fc sites.
   c. Agglutination of antigens via the pentameric structure of IgM allows the cross-linking of antigens.
   d. **Isohemagglutinins, rheumatoid factors,** and **heterophile antibodies** are all IgM.

IN A NUTSHELL

Major features of IgM:
- *First antibody synthesized*
- *Found in B-cell membrane*
- *Pentamer with J chain*
- *Very efficient activator of complement*

3. **IgA** has an important barrier function on mucosal surfaces and functions in the **secretory immune response.**
   a. The serum form is usually monomeric, consisting of two heavy chains (α1, α2) and two light chains.
   b. The **secretory form (sIgA)** is found in tears, colostrum, saliva, milk, and other secretions.
      (1) It is usually dimeric, occasionally trimeric, joined by a polypeptide J chain.
      (2) sIgA contains a 70,000-dalton secretory component (T piece) added to polymeric IgA by epithelial cells as it passes through to the luminal side.
      (3) The secretory component confers stability to the molecule, making it less susceptible to proteolysis in the gastrointestinal tract.
      (4) It is produced by plasma cells in the lamina propria of the gastrointestinal and respiratory tracts.

IN A NUTSHELL

Major features of IgA:
- *Secretory immunity*
- *Often seen as dimer with J chain and secretory piece (sIgA)*

4. **IgD** functions as a cell surface antigen receptor in undifferentiated B lymphocytes. IgD is very susceptible to proteolysis. IgD functions primarily as a receptor for antigen on B cells and stimulates B-cell proliferation. There are no subclasses of IgD.

IN A NUTSHELL

Major features of IgD:
- *Very low concentration in plasma*
- *High levels in membrane of mature B cell*
- *Functions in antigen recognition by B cell*

5. **IgE,** also called the "reaginic antibody," is associated with **allergic response** and **intermediate hypersensitivity.**
   a. The Fc region of IgE binds to the surface of **basophils** and **mast cells.** When antigen cross-links two IgE molecules, mast cells degranulate and release leukotrienes, histamine, eosinophil chemotactic factors, and heparin, which results in an intermediate hypersensitivity reaction. The half-life of IgE is increased when it is bound to cell surfaces.
   b. IgE offers some protection against metazoan parasites, mainly via the release of ECF-A and the induced inflammatory response at the site of worm infestation. Eosinophils degranulate in the area and release major basic protein

IN A NUTSHELL

Major features of IgE:
- *Very, very low concentrations in plasma*
- *Homocytotropic for basophils and mast cells*
- *Reaction with antigen causes release of histamine and production of leukotrienes*

(MBP). This protein has been shown to have a direct toxic effect upon schistosomes and, presumably, acts as a general metazoan poison.

F. **T-cell receptors (TCR)** act as antigen-specific receptors, allowing T cells to function in cell-mediated responses. They do not recognize antigen alone, but only in the context of MHC molecules.

1. Their expression is interwoven with T-cell maturation in the thymus. In the thymus, T cells mature and undergo positive or negative selection. Negative selection deletes autoreactive cells. T cells develop immunologic specificity in a manner similar to that employed by B cells; that is, they use genetic recombination among variable, joining, and diversity genes to achieve their immunologic repertoire. The gene pools are different than those for B-cell (and antibody) diversity, but the processes are very similar.

NOTE

The T-cell receptor has a function of epitope recognition similar to that of an antibody. It also has a structure and genetic composition similar to the Fab portion of antibody nucleus.

G. **Types of immune responses.** Immune responses usually involve some level of inflammation and are an acquired or adaptive response of the body. An extracellular vaccine or pathogen will stimulate a humoral response, while an intracellular pathogen will stimulate both humoral and cell-mediated responses.

1. **Infection at a tissue site causes inflammation,** which enhances antigen delivery by follicular dendritic cells and macrophages to lymphocytes in nearby secondary lymphoid organs and recruits circulating phagocytes and NK cells to attempt to resolve the infection. Activation and clonal expansion of antigen-specific lymphocytes in the secondary organ requires several days to generate a large number of effector lymphocytes and some memory lymphocytes. The effector lymphocytes then circulate through the blood and extravasate at site of infection due to up-regulation of adhesin molecules on endothelial cells.

NOTE

Adjuvants are immunostimulants that increase the immune response by their inflammatory action. They also prolong contact with the immunogen by acting as a depot of antigen deposition.

2. **Humoral immune responses** are generated against most antigens and require the secretion of antibody by plasma cells (activated B cells) in response to a specific immunogenic stimulus.

a. The **primary immune response** is the first response to antigenic exposure. During the initial lag phase, lasting the first 3-5 days, no free antibody can be measured. During this phase, helper T cells and B cells are being activated and activated B cells are differentiating into antibody-secreting plasma cells (the specifics of helper T- and B-cell activation are discussed later). Thereafter, an exponential (log) phase rise in detectable antibody in the circulation occurs.

IN A NUTSHELL

Acquired immunity can be divided into two broad categories:

- ***Humoral, or antibody, responses***
  - *Primary response is always IgM*
  - *Secondary response is a result of isotype or class switching and results in synthesis of IgG, IgA, and/or IgE production*
- ***Cellular responses** involve thymus-derived lymphocytes and culminate in production of helper cells, cytotoxic cells, and delayed-type hypersensitivity cells.*

(1) IgM is primarily produced. After the first IgM response, class switching occurs and the level of IgM usually declines.

(2) Later in the response, higher affinity IgG become detectable; in response to an oral or mucosal antigen, sIgA becomes detectable in secretions as well.

b. The **secondary immune response** is an anamnestic response to previously encountered antigen. Memory B and T cells are responsible for this phase.

(1) In a secondary response, high-affinity IgG class antibody levels rise more rapidly; this requires less antigen to elicit a response. The ability to respond may persist for years due to the presence of long-lived memory B cells and T cells. This anamnestic response explains the efficacy of booster injections of vaccines.

(2) Similarly, a secondary response to an oral antigen rapidly generates higher levels of IgA, much of which is transcytosed across the mucosal epithelial cells onto the external mucosal surface.

(3) Secondary responses to large extracellular particles, such as some helminths, pollens, or enzymes (e.g., those found in cat saliva), may produce unusually high levels of IgE. IgE may be important in the response to helminths, but IgE made in response to pollens and enzymes can lead to allergies.

2. **Cellular immune response.** Effector (activated) helper T cells have multiple functions, including helping activate B cells for most humoral responses, helping activate CTLs, and acting as effector cells in cell-mediated responses such as the killing of virally infected cells and some tumor cells.

a. **Helper T-cell activation** requires presentation of foreign peptides on HLA class II proteins on antigen-presenting cells.

b. Helper T cells secrete cytokines that lead to the differentiation and proliferation of lymphocytes.

c. T cells are the major cell type needed for cell-mediated responses.

(1) Effector $CD4^+$ (helper) cells can secrete cytokines that activate innate cells, e.g., NK cells (to help kill virally infected or tumor cells), or macrophages and neutrophils. Excessive activation of macrophages causes the macrophages to release inflammatory cytokines that can cause delayed-type hypersensitivity.

H. **Regulation.** All immune responses must be regulated. Antigen presentation can affect the strength of the response, as can immune suppression. Anti-idiotypic antibodies may play a role in down-regulating normal humoral responses.

1. **Immunologic tolerance** describes the specific depression of immune responses induced by previous antigen exposure; the opposite of immunity. The degree of immune tolerance may be partial or complete. That is, one may elicit tolerance to one epitope but not to another on a specific molecule. Important factors in tolerance induction include:
   a. **The form of antigen**
   b. **Route of exposure**
   c. **The age of the recipient**.
   d. **Dosage of antigen**

2. **Immune suppression** is described as the active immunologic unresponsiveness resulting from interaction of normal cells with a suppressor cell population or by physical or chemical agent interactions with the immune system.
   a. **Suppression by $CD8^+$ cells.** These cells affect the functions of helper T cells ($CD4^+$), B lymphocytes, and monocytes or macrophages. The mechanism of this suppression is not entirely understood.
   b. **Physical immune suppression**
      (1) **X-ray and ultraviolet radiation** can suppress immune responses by the elimination of lymphocytes. B cells are more sensitive to radiation than are T cells. Bone marrow and lymphoid tissues are especially sensitive.
      (2) **Surgical intervention** can be utilized by the removal of lymphoid tissue such as the thymus, spleen, and lymph nodes.
      (3) **Chemical agents** can successfully suppress immune responses. **Corticosteroids** can cause peripheral blood lymphopenia, inhibit RNA and DNA synthesis, decrease macrophage responses, decrease monocyte chemotaxis, and decrease IgG responses. **Purine or pyrimidine analogs** (e.g., azathioprine) inhibit IgG response. Folic acid **antagonists** interfere with DNA and protein synthesis in lymphocytes. **Alkylating agents** (e.g., cyclophosphamide) reduce the number of lymphocytes in the spleen.

CLINICAL CORRELATE

Some allergies can be controlled by the ingestion of the allergen. This has proven to be of value in some patients with pollen allergies (they eat honey).

CLINICAL CORRELATE

Frequently administered low doses of antigen may be the reason that large organ transplants (e.g., liver) survive well in most recipients.

CLINICAL CORRELATE

A serious consequence of immunosuppression is opportunistic infections. Recurrence of CMV infection in bone marrow transplant recipients is a major cause of morbidity.

CELLULAR INTERACTIONS IN IMMUNE RESPONSES

A. Antigen processing. The mechanism whereby antigen is internalized and re-expressed on the antigen-presenting cell (APC) membrane associated with MHC class I or class II molecules is called **antigen processing.** There are two separate routes that dictate with which MHC molecule the peptide will associate.

1. The **exogenous route** of antigen processing occurs when a cell takes in foreign antigen by phagocytosis or pinocytosis. The antigen is engulfed and is found in endosomes inside the cells. Proteolytic enzymes digest the antigen into small peptide fragments. The endosomes fuse with exocytic vesicles that bear class II molecules on the interior of their membranes and the peptide epitopes then bind in the antigen-binding groove of the **class II MHC.** The endosome then travels to the cellular membrane and fuses with the membrane, thereby expressing the MHC-peptide on the surface of the cell.
2. Small antigens are synthesized within the cell. Examples of such antigens include tumor antigens and viral proteins. These proteins are processed by the **endogenous route** for antigen processing. These proteins are synthesized in the cytoplasm of the cell and some are broken down into peptide fragments. They are transported to the lumen of the endoplasmic reticulum, through the TAP peptide transporter complex, where they associate with **class I MHC molecules.** The MHC I-peptide complex is transported through the Golgi to the cell surface.

B. The T-cell receptor and its interaction with antigen

1. The T-cell receptor is antigen (epitope) specific. It binds to the epitope located in a groove in the appropriate class I or II MHC molecule on the surface of the APC.
2. The TCR is noncovalently linked to the CD3 molecule and engagement of the TCR by antigen stimulates CD3 to transmit biochemical signals into the interior of the cell. These signals are required to trigger the T cell. When peptides are presented in association with MHC molecules, the antigen-binding region of the TCR recognizes processed peptide in association with the MHC molecule. Additionally, the CD4 molecule binds to a different region of the MHC molecule. CD8 molecules can bind to class I MHC, while CD4 molecules cannot. The need for CD4 to bind to MHC II and CD8 to bind to MHC I explains why helper cells ($CD4^+$) respond to processed antigens associated with class II, while cytotoxic T cells ($CD8^+$) respond to antigens associated with class I MHC molecules.

IN A NUTSHELL

- *Endogenous antigens synthesized within the cell, bind with class I MHC molecules, and are presented on the cell surface.*
- *Exogenous antigens are internalized, digested, and fragments are complexed with class II MHC molecules to be presented on the cell surface.*

IN A NUTSHELL

Summary of interleukin role in cell activation:

- *IL-1 produced by macrophages activates Th cells*
- *IL-2 produced by Th1 cells is a signal for proliferation*
- *IL-4,5,6 produced by Th2 cells are signals for maturation and class switching*

IN A NUTSHELL

Interleukins:

IL-1→
- *Stimulates other cells to proliferate, activate, and chemotax*
- *Stimulates IL-2 secretion*
- *Pyrogenic (fever inducing)*

IL-2 →
- *Produced by activated T cells*
- *Stimulates T cells (helper, cytotoxic and natural killer cells)*
- *Stimulates B cells*

IL-3 →
- *Secreted by activated T cells*
- *Stimulates bone marrow stem cells*

IL-4 →
- *Secreted by activated helper cells and mast cells*
- *Stimulates B cells*
- *Increases IgG and IgE*

IL-5 →
- *Secreted by activated helper cells*
- *Promotes B-cell proliferation*
- *Increases IgA and increases synthesis of eosinophils*

IL-6 →
- *Stimulates production of acute phase reactants*
- *Stimulates B cells*

IL-7 →
- *Stimulates pre-B and pre-T cells*

IL-8 →
- *Stimulates chemotaxis and adhesion of neutrophils*

IL-10 →
- *Inhibits cytokine release from macrophages*
- *Inhibits interferon synthesis by Th1 cells*

IL-12 →
- *Activates natural killer cells*
- *Induces Th → Th1*
- *Increases CTL and DTH cell numbers*

C. The **T cell–APC interaction.** We have already discussed the physical interaction of the TCR with the MHC class I or class II-peptide complex. However, for the T cell to become activated, other molecules on the cell surface of the T cell and APC must interact. These accessory molecules are collectively known as **adhesins** and further strengthen the interaction between the T lymphocyte and the antigen-presenting cell.

D. **Requirements for activation of cytotoxic T cells (Tc).** While helper T cells can be fully activated by the T cell-APC interaction, cytotoxic T cells cannot. This is primarily because these cells do not secrete enough IL-2 to support their own growth. For this reason Tc require activated Th1 cells to supply the IL-2 that they need. While the tumor, graft, or virally infected cell itself is able to supply the Tc with peptide associated with MHC class I molecules, antigen-presenting cells must be able to take up some of the soluble antigens and present this antigen to helper T cells. The helper T cells can then supply the IL-2, which is needed by the Tc before it will develop into a fully functional cytotoxic T cell.

E. **Requirements for activation of B cells**

1. When B cells are triggered to make antibody they require the interaction of native (unprocessed) antigen with their antigen receptor (membrane immunoglobulin). However, for class switching to occur, activated T cells must supply necessary Th2 cytokines, including IL-4, IL-5, IL-6, IL-10. B cells also need some IL-2, which may be supplied by a Th1 cell.
2. Some antigens, notably polysaccharides, can stimulate B cells without the need for T cell help. These antigens are said to be "thymus independent." The antibodies made in such responses are IgM only; also, no immunologic memory is induced. This is due to the fact that helper T cells do not respond to polysaccharides since they cannot be processed.
3. Protein antigens are "thymus dependent." Immunoglobulins made to thymus-dependent antigens will include all classes of immunoglobulin since class switching will occur in the responses due to the presence of Th2-derived cytokines.

F. **Cytokines.** Cytokine is a term used to describe a collection of proteins that regulate immunologic and inflammatory response to injury. **Lymphokines** are cytokines produced by lymphocytes. **Monokines** are cytokines produced by monocytes and macrophages. Cytokines specifically modulate responses to antigen in several ways, including the regulation of cell growth and the control of differentiation of immunologic cells. Cytokines mediate intercellular signalling that controls activation or

pression of immune cells. Normally, resting cells do not secrete cytokines; cells must be stimulated to produce them. Cytokines have both **autocrine effects** (i.e., exerts a local effect on the same cell that produces the cytokine) and/or **paracrine effects** (exerts an effect over a distance upon other cells). Cytokines are typically very potent agents that can act on their target cells at very low concentrations. Also, a single cytokine can have multiple functions and many cytokines have similar effects. Cytokines often influence the synthesis of other cytokines, leading to cascades with both positive and negative regulatory mechanisms.

COMPLEMENT AND INFLAMMATION

Inflammation is an integral part of all immune responses. One of the major mechanisms for initiating inflammation is activation of the complement cascade, which also produces powerful opsonins, chemoattractants, and anaphylatoxins, and can directly mediate killing through cell lysis.

A. **Complement.** Historically, complement was the term used to describe the activity in serum which, when combined with specific antibody, would cause bacterial lysis. Complement is now known to be a system of proteins that interact to play a role in humoral immunity and inflammation. Complement is a complex group of proteins and glycoproteins found in blood and tissue fluids. Complement can directly opsonize foreign material for phagocytosis after antibody activation (C3 component) and can participate directly in killing some cells and microorganisms. Finally, peptide fragments from the complement proteins can regulate inflammation.

1. **Complement activation** (see Figure 1-5). There are two pathways of complement activation, the **classical pathway** and the **alternative pathway.** The end result of either pathway is the formation of a **membrane attack complex (MAC),** which is a lipid-soluble pore structure that causes osmotic lysis of cells.
   a. The **classical pathway** is the main antibody-directed mechanism for complement activation. It is the most rapid and efficient pathway.
      (1) Complement recognizes antigen-antibody complexes. The antibody must be **IgG or IgM,** but IgM is more efficient than IgG.
      (2) Antigen-antibody complexes bind to C1, which then activates the cascade.
   b. The **alternative pathway** of complement activation occurs as a result of binding directly to the surface of an infectious organism. This pathway is slow and less efficient than the classical pathway.

CLINICAL CORRELATE

The alternative pathway protects the body from pathogens in the absence of antibody. The bacterial surface itself activates the cascade.

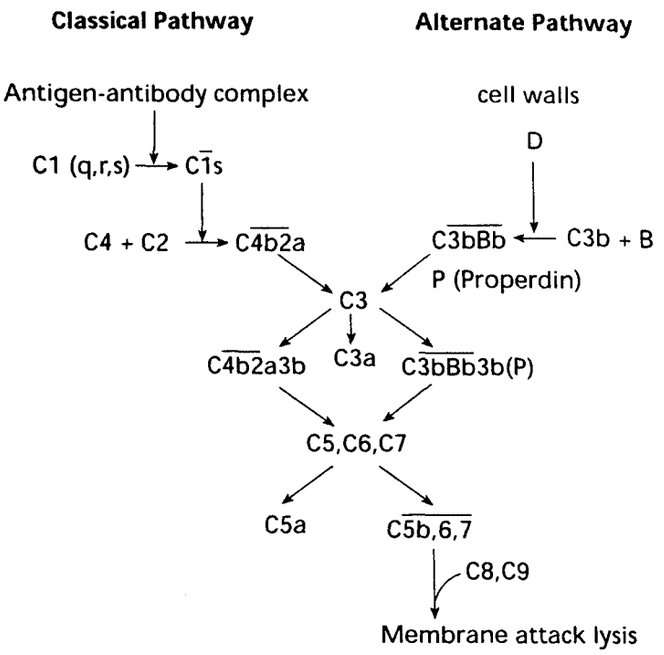


Figure 1-5. The complement cascade.

(1) The alternative pathway primarily recognizes bacteria and depends upon the interaction of a small amount of preformed C3b with protective surfaces.

(2) It is important to remember that the two pathways both lead to lysis by the terminal complement components (C8 and C9); however, they are initiated by different complement components.

2. **Classical pathway.** The reaction occurs in the following order: **C1q, C1r, C1s, C4, C2, and C3**. Each component of the classical pathway has unique biological functions. (See Figure 1-5)

3. **Alternative pathway.** There are several key components of the alternative complement pathway. (See Figure 1-5)
   a. There are several initiators of the alternative pathway.
      (1) Lipopolysaccharides, the cell wall components of Gram-negative bacteria
      (2) Bacterial and plant polysaccharides
      (3) Cell membrane constituents
      (4) Aggregated IgA, IgG, IgE, and IgM
      (5) Cobra venom factor
      (6) Endotoxins that complex with Factor B to form C3 convertase

4. **The membrane attack complex (MAC)** is formed from the interactions among C5, C6, C7, C8, and C9. It leads to lysis of cells.

IN A NUTSHELL

Biologically important C proteins:
- *C2a, C4a = weak anaphylatoxins*
- *C3a, C5a = strong anaphylatoxins*
- *C5a = potent chemotaxin*
- *C3b = potent opsonin*

5. The products of the complement cascades have several biological roles including viral neutralization, lysis of infected cells, and direct pathogen lysis. Complement activation can also mediate immune adherence, the focusing of antigen on macrophage or lymphocyte surfaces, and promoting phagocytosis. Finally, biological responses such as anaphylaxis, kinin activity, smooth muscle contraction, vasodilatation, and chemotaxis are mediated by individual complement components.

Inflammation is a pathologic state initially characterized by **pain, redness, heat,** and **swelling.** These features of inflammation are due to vascular permeability changes leading to an infiltration of leukocytes, primarily neutrophils and macrophages, although small numbers of eosinophils and basophils can also be found. Lymphocytes can also enter sites of inflammation, where the release of cytokines can enhance the inflammatory response. A variety of compounds or chemicals can elicit inflammatory responses, and a variety of chemotactic factors are responsible for the migration of cells to sites of inflammation.

1. **Vasoactive and smooth muscle constrictors**
   a. **Histamine**
      (1) Histamine is stored as granules in **mast cells, basophils,** and **platelets,** with higher concentrations found in the intestine, lung, and skin.
      (2) Histamine is released from mast cells when antigen contacts IgE bound on the mast cell. It may also be released by nonimmunologic mechanisms, e.g., trauma or cold.
      (3) Histamine interacts with target cell receptors H1, H2, and H3. H1 causes contraction of smooth muscle, increases vascular permeability, and elevates intracellular cyclic GMP. H2 increases gastric acid secretion, respiratory mucus production, and intracellular cyclic AMP. H3 is found in the central nervous system and functions in the negative feedback inhibition of histamine release and synthesis.
      (4) Histamine can be isolated from inflammatory sites in early inflammation, but its concentration dwindles within 1 hour.
   b. **Arachidonic acid products.** Arachidonic acid is derived from cell membrane phospholipids, following conversion from linoleic acid. It is degraded via two pathways, the cyclooxygenase pathway and the lipoxygenase pathway.
      (1) The **cyclooxygenase pathway** converts arachidonic acid to prostaglandin PGG_2. PGG_2 is converted to PGH_2, which is ultimately converted to TxA_2 (thromboxane

CLINICAL CORRELATE

Deficiencies in complement components predispose patients to certain diseases:

C3 deficiency	→	*Increased susceptibility to pyogenic infections*
C2 deficiency	→	*Increased incidence of connective tissue disorders*
C5-8 deficiency	→	*Recurrent Neisseria infections (meningococcal, gonococcal)*
C1 esterase inhibitor deficiency	→	*Hereditary angioneurotic edema*

IN A NUTSHELL

The inflammatory response can be triggered by local tissue damage that results in enzyme activation, or by mast cell degranulation caused by anaphylatoxin interaction with specific receptors (e.g., C3aR, C5aR) on the cytoplasmic membrane. Allergen reacting with cell-bound IgE can also trigger degranulation.

A_2), other more stable prostaglandins ($PGF_2\alpha$, PGE_2, PGD_2), and prostacyclin ($CPGI_2$).

(2) The **lipoxygenase pathway** produces the **leukotrienes** including LTC_4, LTD_4, and LTE_4, which are collectively known as the **slow-reacting substance of anaphylaxis.**

c. **Platelet-activating factor (PAF)** is derived from cell membrane lipid and is synthesized by basophils, neutrophils, monocytes, and epithelium.

(1) PAF activates platelets by initiating the release of platelet granule constituents, causing platelets to clump.

(2) PAF stimulates the synthesis of prostaglandins and leukotrienes.

(3) PAF also increases the adhesiveness of neutrophils for endothelial cells.

d. **Adenosine** is an inflammatory agent derived from mast cells after ATP breakdown. It interacts with A1 and A2 receptors on the cell membrane. The A2 receptor is associated with increased levels of intracellular cyclic AMP, an effect that is blocked by methylxanthine drugs.

2. **Chemotactic factors**

a. **Eosinophil chemotactic factors.** There are six major eosinophil chemotactic factors including histamine.

b. **Neutrophil chemotactic factors.** There are six neutrophil chemotactic factors. Their primary role is to attract neutrophils to sites of inflammation. They include IL-1 and IL-8.

3. **Enzyme mediators**

a. **Neutral proteases** are products of mast cells.

b. **Acid hydrolases** are found in the lysosomes of many cell types. They degrade membrane components such as chondroitin sulfate.

4. **Proteoglycans** function in the storage and release of other vascular mediators.

a. **Heparin** is an anticoagulant that modulates tryptase activity. **Tryptase,** the major protein of human lung mast cells, can activate C3 directly in the absence of heparin. It is stored in mast cell granules in association with histamine.

b. **Chondroitin sulfates** are structural molecules that function as binding sites for other mediators within mast cells.

5. **Toxic oxygen molecules** are important compounds that function in killing microorganisms and in the activation of neutrophils, eosinophils, and mast cells. They include singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radicals.

CLINICAL CORRELATE

Eosinophils are important cells of the innate immune system; they augment acquired immunity to metazoans. They contain basic proteins in their granules that are toxic to worms.

6. **Kinins** are polypeptides (e.g., fibrin-split peptides) formed from precursors in the plasma.
   a. Kinins are labile proteins with physiologic effects similar to histamine.
   b. **Bradykinin,** a major kinin, functions as a vasodilator by increasing capillary permeability and producing erythema and edema. It also causes smooth muscle contraction.

CLINICAL CORRELATE

Enzyme activation during the complement cascade or as a part of the inflammatory response can activate the clotting system (Hageman factor, etc.) and lead to DIC.

Clinical Immunology

This chapter discusses immunopathology, transplantation, and tumor immunology. Immunopathology includes two broad areas: immunodeficiency states and autoimmune or hyperimmune reactions.

IMMUNODEFICIENCIES

In immunodeficiency, there is a failure to produce cellular immunity, humoral immunity, or both. Several genes have been identified that cause congenital immune deficiency. Acquired immune deficiency may be due to human immunodeficiency virus 1 and 2 (HIV 1, HIV 2), or may be iatrogenic when patients are treated with steroids, radiation, or chemotherapy. Some diseases (particularly lymphomas) cause immunodeficiency because they involve the cells and organs of the immune response. In view of the complex nature of the immune response, it is not surprising that a wide array of deficiencies exist, heralded primarily by recurrent infection, chronic infection, unusual (opportunistic) infecting agents, and a poor response to treatment. Occasionally, other manifestations, such as hepatosplenomegaly and diarrhea, are seen. When an immunodeficiency syndrome is suspected, the workup of the patient includes an evaluation of the native and acquired immune capabilities. These assays include an analysis of phagocytic functions (chemotaxis, phagocytosis, and killing), complement levels, antibody production, and cell-mediated immunity.

A. **Primary immunodeficiency diseases** represent defects in selected members of the immune system. With few exceptions, the primary biologic error for these defects is not known. Immunodeficiency diseases occur more frequently in children than in adults (3:2), with male:female ratio of 5:1.

CLINICAL CORRELATE

Most major opportunistic pathogens are normal flora agents like Staphylococcus, Candida, etc. Environmental agents include water bacteria such as Pseudomonas and Legionella and fungi such as Aspergillus and Rhizopus.

1. **Antibody deficiency disorders** are congenital or acquired, partial or total, and are typically recognized because of recurrent opportunistic bacterial infections or treatment failure.
   a. **X-linked (Bruton's) agammaglobulinemia**
      (1) **Etiology.** Usually arises in males after 6 months of age, as this is the time when maternally derived immunoglobulins have totally disappeared. It arises from a failure of B-cell maturation, resulting in the absence of immunoglobulins, plasma cells, and germinal centers of lymphoid tissue. Cell-mediated immune responses are generally intact.
      (2) **Clinical features.** The clinical symptoms include repeated infections with extracellular pyogenic organisms and also increased enteroviral infections. Pneumococcal, other streptococcal, and Haemophilus infections are most common. Infections include pneumonia, sinusitis, meningitis, furunculosis, otitis, septicemia, conjunctivitis, echovirus, and encephalitis.
      (3) **Therapy.** Replacement therapy with **parenteral immune serum globulin** is the usual treatment.
   b. **Common variable (B lymphocyte) hypogammaglobulinemia.**
      (1) **Etiology.** It is a congenital or acquired syndrome with equal sex distribution. The defect may appear at any age and is in the abnormal terminal differentiation of B cells. Bacterial infections predominate but are less severe than in the X-linked form. Immunoglobulin-bearing B lymphocytes are normal in number, but **serum antibody deficiency is profound** as B cells proliferate but fail to differentiate into plasma cells.
      (2) **Clinical features.** Tonsils and lymph nodes are normal-sized or enlarged and splenomegaly is common. Clinical presentations include alopecia areata, thymoma, lymphoreticular malignancy, hemolytic anemia, gastric atrophy, and achlorhydria. There is also a high incidence of pyogenic infections, autoimmune diseases, and malabsorption.
      (3) **Treatment** is with replacement immune gamma globulin.
   c. **Selective IgA deficiency** is the most common immunodeficiency state (greater than 1/1,000 population), particularly in individuals of European descent.
      (1) **Etiology.** The disease is marked by reduced levels of serum and secretory IgA. IgA deficiency is thought to be caused by defective class III MHC gene(s) on chromosome 6.

CLINICAL CORRELATE

Bruton's disease is approximately 100× less frequent than selective IgA deficiency, but clinical disease is almost a certainty with Bruton's.

(2) **Clinical features.** The majority of patients appear healthy, but may have increased incidence of respiratory infections. IgA deficiency patients are predisposed to certain diseases including atopy, autoimmune disorders (such as SLE, pernicious anemia, and rheumatoid arthritis), and certain malignancies.

2. **Cellular immunodeficiency disorders**
   a. **Thymic hypoplasia or aplasia (DiGeorge's syndrome)** is a condition due to the dysmorphogenesis of the third and fourth pharyngeal pouches, leading to hypoplasia or aplasia of the thymus and parathyroid glands, and congenital heart disease. Immunoglobulin concentrations are usually normal but may be elevated. T-cell levels are decreased. Patients are prone to viral and fungal infections.
   b. **Cellular immunodeficiency with immunoglobulins (Nezelof's syndrome)** presents clinically with lymphopenia, abnormal thymus, and reduced lymphoid tissue, but with no cardiac or endocrine abnormalities. Serum immunoglulubulins are normal or elevated. The patient usually presents with recurrent or chronic pulmonary infections, oral or cutaneous candidiasis, chronic diarrhea, Gram-negative sepsis, or severe varicella. This syndrome can be distinguished from pediatric AIDS patients in a number of ways: patients with Nezelof's syndrome usually have normal helper T-cell suppressor ratios, the peripheral lymphoid tissue is hypoplastic, and the thymus is small.
3. **Severe combined immunodeficiency (SCID)** is a group of disorders with defects in humoral and cell-mediated immunity, usually due to **defects in lymphoid stem cells.** The incidence is 1:100,000 births. The thymus gland lacks lymphoid cells as do peripheral lymphoid organs such as the spleen, Peyer's patches, etc. Patients usually succumb to infection within the first year. There are two modes of inheritance.
   a. **Autosomal recessive (Swiss-type agammaglobulinemia)** is the congenital absence of both cellular and humoral immune function due to severe T and B cell lymphopenia. Opportunistic infections lead to death. This syndrome may be associated with lymphopenia and hypoplasia of the thymus gland. There is an absence of T cells and an inability to mount a humoral immune response.
   b. **X-linked recessive is the most common SCID** (sometimes abbreviated XSCID). The disease clinically resembles the autosomal recessive form, but no severe leukopenia occurs. Most patients have normal numbers of B cells with few or no circulating T cells.

IN A NUTSHELL

Bruton's agammaglobulinemia:
- *X-linked (males)*
- *Pre-B cells are normal but B cells are absent*
- *Low circulating antibody levels*
- *Normal cell-mediated immunity*
- *Recurrent bacterial infections*

Common variable hypogammaglobulinemia:
- *Equal sex distribution*
- *B cells normal but don't differentiate to plasma cells*
- *Low circulating antibody levels*

Selective IgA deficiency:
- *Low levels of IgA*
- *Present with respiratory infections; milk allergies common*

CLINICAL CORRELATE

Children with congenital infections often suffer a wasting syndrome and have general immunodeficiency. The agents include:
- *Toxoplasma*
- *Rubella*
- *Cytomegalovirus*
- *Herpes simplex, HIV*
- *Syphilis (Treponema)*

These are called the ToRCHeS agents.

CLINICAL CORRELATE

Children with Down's syndrome (chromosome 21 trisomy) have a 100-fold higher mortality from respiratory infections than normal. They have thymic hypoplasia and phagocytic defects but normal B-cell functions.

IN A NUTSHELL

Hyper-IgE syndrome:

- *IgE = > 3,000 Iμ/ml*
- *↓ Antibody responses*
- *↑ Respiratory infections, especially S. aureus*
- *Eosinophilia*
- *Dermatitis*
- *Growth retardation*

B. **Acquired immunodeficiency**. Acquired immunodeficiency syndrome is the defining infectious disease of our generation. AIDS is transmitted by contact with blood or other body fluids of an infected individual.

1. **Causative agent.** AIDS is caused by the **human immunodeficiency virus (HIV),** which is a C-type retrovirus belonging to the Lentivirus family. The RNA core is surrounded by a lipid envelope that is derived from the host plasma membrane. The viral membrane contains a transmembrane protein, gp160. This protein is commonly detected in Western blot analysis as two fragments—gp41 and gp120. The core proteins include **reverse transcriptase** and two nonglycosylated proteins designated p18 and p24.
2. **Risk groups** for HIV infection include homosexual males, intravenous drug abusers, children born to infected mothers, sexual partners of people in high risk groups, and recipients of infected blood products or secretions. The latter risk is now minimal given the excellent methods of screening blood products for potential contamination.
3. **Mechanism of transmission.** Modes of transmission of HIV include needle contamination among drug abusers, sexual contact, and exposure to blood during the birth process.
4. **Mechanism of infection.** AIDS is characterized by a **profound loss of $CD4^+$ T cells.** The virus binds to the CD4 molecule on the T cell via gp120. In addition to T cells, HIV can also infect other $CD4^+$ cells, such as macrophages and astrocytes in the brain. Having entered into the cell, the RNA is reverse transcribed and integrated within the host DNA. Exposure of the T cell to activating stimuli such as cytokines or antigens results in activation of the virus by stimulating transcription of virally encoded genes. Once activated, the virus replicates within the cell. Extensive viral budding can lead to death of the T cell. In contrast to the T cell, the macrophage is more resistant to death from viral infection and appears to serve as an important viral reservoir.
5. **Cellular consequences of HIV infection**
   a. **T cells.** A central consequence of infection with HIV is a loss of $CD4^+$ helper T cells. However, in addition to a loss of $CD4^+$ T cells, there is also a decrease in the response of T cells to antigen, and impaired production of cytokines, such as IL-2 and IFN-γ.
   b. **B cells.** Although patients with early HIV infection appear to have polyclonal activation of B cells with circulating immune complexes in their plasma, they steadily lose th

NOTE

The loss of $CD4^+$ cells would lead one to anticipate a deficit in helper cell activity with reduced antibody synthesis, but this may not occur. The pathogens that predominate (i.e., fungi, viruses, and mycobacteria) in these patients are consistent with a T-cell defect.

ability to mount an effective antibody response to new antigens.

c. **Macrophages.** HIV can enter the macrophage through binding of gp120 to CD4 and a second membrane receptor, CCR5 (a chemokine receptor). Both circulating monocytes and macrophages serve as a reservoir for the virus.

6. **Natural history of HIV infection**. The Centers for Disease Control (CDC) has proposed four clinical subgroups for AIDS-infected individuals:
   a. **Group I.** This group of individuals is characterized by an acute infection. After the initial infection, the patient may develop a syndrome resembling infectious mononucleosis, characterized by rash, sore throat, fever, or even aseptic meningitis.
   b. **Group II.** This group of individuals is characterized by asymptomatic infection. The disease becomes clinically latent and can remain so for 7-10 years.
   c. **Group III.** This group is characterized by persistent generalized lymphadenopathy together with fever, rash, and fatigue. **AIDS-related complex (ARC)** represents a nonspecific cluster of signs and symptoms of AIDS that is not accompanied by a decrease in $CD4^+$ cells. A diagnosis of early ARC is made if the individual has one or two of the following symptoms: fatigue, fever, weight loss, persistent skin rash, oral hairy leukoplakia, herpes simplex, and oral thrush. Advanced ARC is determined if the individual has two or more of these symptoms.
   d. **Group IV.** This group is characterized by generalized disease, including neurologic opportunistic infections and secondary neoplasms. Ultimately, the disease progresses to the breakdown of the immune system with full-blown AIDS characterized by the development of secondary tumors and numerous opportunistic infections.
      (1) Among the tumors common to AIDS is **Kaposi's sarcoma,** which can be found in individuals even before breakdown of the immune system.
      (2) NonHodgkin's lymphomas are increasingly common tumors that are found in severely immunocompromised patients.
      (3) HIV-infected individuals are also susceptible to opportunistic infection by protozoa (e.g., Cryptosporidia and *Toxoplasma gondii*), fungi (e.g., Cryptococcosis, Candidiasis, and *Pneumocystis carinii*), bacterial (e.g., *Mycobacterium avium-intracellulare* and *M. tuberculosis*),

CLINICAL CORRELATE

HIV patients can present with a wide range of problems across organ systems. If you encounter an HIV (or high-risk group) patient on the exam, you should know that there is a high incidence of the following disorders in HIV patients:

- *Oral thrush (Candida)*
- *Esophagitis (Candida, CMV, HSV)*
- *Diarrhea*
  - *Bacterial (Salmonella, Shigella, Yersinia, Campylobacter, Myobacterium avium-intracellulare, C. difficile)*
  - *Parasitic (Cryptosporidium, Isospora, Giardia, Entamoeba)*
  - *Viral (CMV colitis)*
  - *Fungal (Candida)*
- *Intestinal neoplasms: lymphoma, Kaposi's sarcoma*
- *Pneumonias (especially Pneumocystis carinii)*
- *Tuberculosis and atypical mycobacterial infection*
- *Fungal respiratory disorders: histoplasmosis, coccidioidomycosis*
- *Pulmonary neoplasms: Kaposi's sarcoma, lymphoma*
- *Hematologic problems: anemia, leukopenia, thrombocytopenia*
- *Neurologic disorders*
  - *Cryptococcal meningitis*
  - *Toxoplasmosis*
  - *Progressive multifocal leukoencephalopathy*
  - *CMV encephalopathy*
  - *CMV retinitis*
  - *AIDS dementia*
  - *CNS lymphoma*
- *Sexually transmitted diseases*
- *Skin*
  - *Shingles*
  - *Kaposi's sarcoma*
  - *Seborrheic dermatitis*
  - *Herpes simplex*
- *Disseminated infections*
  - *Cytomegalovirus*
  - *Mycobacterium avium-intracellulare*
  - *Histoplasmosis*

and viruses (e.g., cytomegalovirus [CMV], herpes simplex, and varicella zoster).

(4) Central nervous system (CNS) involvement is a common feature of AIDS (60%). It can result either from direct infection of the CNS by the virus or from opportunistic infections (e.g., toxoplasmosis).

7. **Diagnosis.** During primary illness, antibodies are not commonly detected. HIV antibodies usually appear six weeks to six months after exposure. Antibodies against the various viral proteins do not develop simultaneously. For example, antibodies against p24 (core) and gp41 (transmembrane proteins) are detected before those induced by pol gene products.
   a. Antibodies to HIV are detected by ELISA.
   b. Confirmatory tests are performed by Western blot.

8. **Treatment, prognosis, and prevention.** While the average incubation period for infected adults is 10+ years, infants develop clinical disease much sooner after exposure. The treatment is aimed at retarding viral replication and preventing opportunistic infection. Patients with AIDS or AIDS-related complex (ARC) are treated with **nucleoside analogs,** such as zidovudine (3'-azido-3'-deoxythymidine or AZT) and with **protease inhibitors** (e.g., saquinavir). The most important treatment at this time is **prevention,** whether through education, counseling, or behavior modification. In addition, screening of potentially infected blood has virtually eliminated transmission by blood transfusion, and cloned antihemophilic factor (factor VIII) has reduced the risk of HIV transmission to hemophiliacs.

C. **Phagocytic cell disorders** are characterized by a heightened incidence and severity of infections caused by bacteria, particularly pyogenic organisms such as Staphylococci and Streptococci. They may be classified into syndromes defective in the production and/or maturation of cells (production defects) or syndromes where cells are present but functionally aberrent (functional defects).

1. **Cyclic neutropenia** is defined as a periodic neutropenia, usually occurring every 21 days, with corresponding intermittent bone marrow maturation arrest at the promyelocyte stage.
2. **Hereditary neutropenia** is usually fatal by age 1. It is characterized by the absence of peripheral blood neutrophils from birth and by the arrested maturation of bone marrow precursors.
3. **Acquired neutropenia** has several known causes, including:
   a. Hyposplenism (as seen in sickle cell disease)

BRIDGE TO PHARMACOLOGY

These drugs are discussed in greater detail in the chapter on Antimicrobial Agents in this book.

b. Neoplasms, particulary myelocytic leukemia and metastatic carcinoma
c. Overwhelming infection from tuberculosis, typhoid fever, measles, infectious mononucleosis, and leishmaniasis
d. Drug toxicity from many cancer chemotherapeutic agents, antibiotics, and diuretics
e. Irradiation
f. Hemodialysis and cardiopulmonary bypass patients

4. **Opsonic defects** are functional defects of phagocytic cells commonly due to the lack of serum factors, primarily complement products. Opsonization refers to the clearance of organisms (or other target cells) via antibody or complement attaching to the target organism and by the engulfment of this complex following attachment to complement or Fc receptors.

5. **Chemotactic defects** refer to the impaired ability of cells to respond or move toward a chemical stimulus. As described above, cells of the lymphoid system can normally migrate toward a variety of stimuli, such as cytokines and mediators of inflammation. Defects in chemotaxis can be due to serum inhibitors, possibly associated with alcoholic liver disease, recurrent staphylococcal infections, Hodgkin's disease, or agammaglobulinemia. In addition, drugs (steroids, colchicine) or C5 defects can affect normal chemotaxis. Several disease states are associated with killing defects, including glucose-6-phosphate dehydrogenase deficiency (G6PD) and myeloperoxidase deficiency. Several syndromes are associated with impaired cellular chemotactic responses, including Chédiak-Higashi syndrome, lazy leukocyte syndrome and hyperimmunoglobulinemia E with impaired chemotaxis (Job's syndrome).

HYPERSENSITIVITIES

Hyperimmune reactions cause disease syndromes when the normal protective functions of immunity become imbalanced. Excessive immunoglobulin E (IgE) can lead to allergy; antigen-antibody (Ag-Ab) complexes can provoke arteritis, arthritis, and glomerulonephritis; and cytotoxic antibodies can destroy red blood cells (RBCs), white blood cells (WBCs), platelets, or, less frequently, other cells in the body. Inappropriate or undesirable cell-mediated immunity can lead to graft rejection or demyelinating disease. There are four distinct types of hypersensitivity reactions characterized by the time course required for the induction of the response, the immune cells and soluble factors involved, and the types of antigen involved.

IN A NUTSHELL

Immune deficiency	***Predominating opportunist***
B cell	*Pyogenic bacteria*
T cell	*Viruses and fungi; also mycobacteria*
C3	*Pyogenic bacteria*
C5-8	*Neisseria*
Phagocytes	*Pyogenic bacteria*

NOTE

These reactions are called "immediate" because symptoms of the allergy will be seen in a patient 10-20 minutes after exposure to the allergen. There must have been a previous antigenic encounter to induce the sensitivity. Always think of type I (atopic, IgE mediated) hypersensitivity when the symptoms (rash, wheezing, cramps) occur rapidly after antigen injection/ingestion.

A. **Type I hypersensitivity** (or immediate, atopic or anaphylactic hypersensitivity) requires an initial exposure to antigen in order to sensitize the person. Re-exposure to the same antigen causes cross-linking of IgE receptors on the surface of basophils and mast cells (via Fce receptors). The mast cells then release a variety of pharmacologic mediators. The systemic reactions occur within minutes of secondary exposure to the allergen. Smooth muscle contraction leads to constriction of bronchi and bronchioles while vasodilatation and increased vascular permeability results in peripheral edema. The significant cutaneous response is a wheal and flare reaction ("hives" or urticaria). The typical clinical syndromes associated with type I hypersensitivity include asthma, atopic dermatitis, eczema and allergic rhinitis (hay fever).

1. The important preformed mediators (and their primary biologic functions) released from basophils and mast cells upon the cross-linking of IgE are:
   a. Histamine (smooth muscle contraction and vasodilatation)
   b. Heparin (anticoagulant)
   c. Eosinophil chemotactic factor of anaphylaxis (ECF-A) (chemotaxis)
   d. Platelet-activating factor (PAF; microthrombi and platelet degranulation with release of vasoactive compounds)
   e. Tryptase (activates C3; inhibited by heparin)
2. In addition, several biologically active compounds are newly synthesized during mast cell degranulation and the ensuing inflammatory response. These include compounds formed from the arachidonic acid breakdown product of cell membrane phospholipids (via phospholipase A2, which is activated during Ca^{2+} influx that accompanies IgE cross-linking by allergen):
   a. **Leukotrienes (LT)** are formed from arachidonic acid via the lipoxygenase pathway.
   b. **Prostaglandins (PG)** are formed via the cyclooxygenase pathway from arachidonic acid.
   c. The **anaphylatoxins C3a and C5a** are generated via tryptase action on the native complement proteins; they cause mast cell degranulation by reacting with C3a and C5a receptors in the cell membrane (anaphylactoid reactions).
   d. **Bradykinin** is generated from kininogen by the action of kallikrein, activated Hageman factor (factor XIIa), or trypsin; it stimulates vasodilatation and increases vascular permeability.
3. Common allergens that induce type I or immediate responses include:
   a. Foods (milk, eggs, fruits, and shellfish)

b. Pollen
c. Drugs (e.g., penicillin)
d. Insect venom
e. Animal dander
f. House dust, mite fecal pellets

4. **Anaphylaxis** refers to an immediate hypersensitivity response that is inducible in a normal host of any given species upon appropriate antigenic exposure (called sensitization). Anaphylaxis of type I hypersensitivity occurs rapidly and may be life-threatening. Victims may die of asphyxiation due to respiratory tract edema and bronchial constriction. **Atopy** refers to an immediate hypersensitivity response that occurs only in genetically predisposed hosts upon sensitization to specific allergens. This condition differs from anaphylaxis in that it cannot be induced in normal hosts. As in anaphylactic reactions, the response in atopic reactions is characterized by smooth muscle contraction and increased capillary permeability with edema.

5. **Treatment of type I hypersensitivity**
   a. **Avoidance.** The most direct way to manage allergic disease is through environmental control.
   b. **Hyposensitization** is a form of immunotherapy aimed at stimulating the production of **IgG blocking antibody**, which binds the offending allergen and prevents its combining with IgE on the mast cell.
   c. **Desensitization** may be produced by a series of closely spaced, small injections of allergen. This "uses up" the IgE in the body without causing significant symptoms. Once the desensitizing injections are discontinued, the hypersensitivity will return when sufficient allergen-specific IgE is generated to sensitize the basophils and mast cells. This treatment has been used to desensitize patients to particular antibiotics that are required for therapy of their infection.
   d. **Drug treatment** involves administration of agents designed to:
      (1) Block mediator binding to target tissue (e.g., antihistamines)
      (2) Stabilize granule and/or cell membranes (e.g., corticosteroids). In addition, corticosteroids such as prednisone increase beta receptor numbers in tissues and inhibit phospholipases, thus blocking mediator synthesis and secretion. These compounds also interfere with cell adherence to the vascular endothelium and diapedesis.

NOTE

Anaphylactoid reactions do not require presensitization. They will occur shortly after a first-time encounter with a material that can activate the alternative complement pathway and cause mast cell degranulation or activate arachidonic acid metabolism.

(3) Stimulate adenyl cyclase, the enzyme responsible for conversion of adenosine triphosphate (ATP) to cAMP and, thereby, block the release of mediators (e.g., epinephrine).

(4) Block the release of mediators by stabilizing mast cell membranes and inhibiting Ca^{2+} influx (e.g., cromolyn sodium).

(5) Inhibit phosphodiesterase, an enzyme that converts cAMP to AMP, thus preserving levels of cAMP essential for blockage of mediator release (e.g., theophylline).

B. **Type II hypersensitivity,** or antibody-mediated cytotoxicity may be associated with autoimmune diseases. IgG or IgM antibody reacts with membrane-associated antigen on the surface of cells, causing the activation of the complement cascade and, ultimately, cell destruction. Alternatively, natural killer cell-mediated lysis (antibody-dependent cellular cytotoxicity; ADCC) of an antigen-bearing target cell may occur. The response may be transient or chronic.

1. **Complement-mediated cytotoxicity** results when IgM or IgG reacts with antigen, resulting in activation of the complement system and phagocytosis or lysis of cells where complement has been fixed.
2. **Antibody-dependent cell-mediated cytotoxicity (ADCC)** results when cells with Fc receptors (especially NK cells, but also monocytes and neutrophils) bind to target cells coated with IgG, causing phagocytosis or lysis of target cells.
3. **Examples of type II reactions**
   a. Certain drug allergies
   b. Blood transfusion reactions (red cell lysis)
   c. Hemolytic disease of the newborn
   d. Goodpasture's syndrome with antiglomerular basement membrane antibody formation
4. **Therapy for cytotoxic reactions** includes treatment of the underlying cause of the reaction as well as the subsequent manifestations of such a reaction. Examples of therapeutic measures include:
   a. Suppression of the immune response by means of corticosteroids, with or without cytotoxic immunosuppressive drugs (e.g., cyclophosphamide).
   b. Removal of offending antibodies via exchange transfusion (in the case of Rh hemolytic disease of the newborn) or plasmapheresis.
   c. Withdrawal of the offending allergen in the case of drug-induced syndromes.

CLINICAL CORRELATE

There are several autoimmune diseases that may be considered type II hypersensitivities even though the antibody is not "toxic." For example, in Graves' disease, the antibody actually causes hyperthyroidism. Also anti-B_{12} antibodies cause pernicious anemia.

C. **Type III hypersensitivity,** or immune-complex-mediated hypersensitivity, is caused by antibodies formed to foreign antigens (typically, horse antibodies to snake venom or diphtheria toxin), or by antoantigens such as DNA. It occurs only with complement-fixing antibodies (i.e., IgG and IgM). Immune complexes of IgG or IgM with horse gamma globulin activate complement, resulting in the generation of C3b, which promotes neutrophil adherence to blood vessel walls. Immune complexes also generate the anaphylatoxins C3a and C5a that lead to inflammation and tissue destruction. The hallmark signs of sickness become apparent at 7-14 days and include urticaria, angioedema, fever, chills, and malaise.

1. **Serum sickness** is the hallmark syndrome of type III hypersensitivity. It results from immune complex deposition in small vessels. Serum sickness may improve quickly or persist for months depending on the type of antigen and the levels of antibody. Drug hypersensitivity reactions to penicillin, streptomycin, sulfonamides, and phenylbutazone may also cause serum sickness.
2. The **Arthus response** is the cutaneous reaction of type III responses. The Arthus response is highly localized (in and around blood vessels), appears within an hour, and resolves within 12 hours unless there has been severe tissue necrosis. The Arthus response is easily demonstrated in animal models. The repeated immunization of the animal results in high levels of antibody (usually IgG). The antigen is then injected intradermally or subcutaneously and complexes locally with antibody. The complement cascade is activated and the reaction site becomes edematous and hemorrhagic. The intravascular clumping of platelets may lead to tissue necrosis.
3. Immune complexes are the central elements in type III pathologic events. Tissue deposition is most likely triggered by increased vascular permeability caused by vasoactive substances described earlier. In autoimmune disease, immune complexes are formed by autoantibodies (such as anti-DNA in SLE) with its antigen. Immune complexes may then bind preferentially to certain tissues. In SLE, anti-DNA immune complexes bind to kidney glomeruli, causing nephritis. In rheumatoid arthritis, rheumatoid factor immune complexes may be found in joint spaces.
4. **Clinical features** include urticaria, lymphadenopathy, edema, and fever. Occasionally, arthritis, vasculitis, and glomerulonephritis are seen. The hallmark of immune complex glomerulonephritis is its granular ("lumpy bumpy") appear-

ance as detected by immunofluorescense using tagged antibody specific for immunoglobulin or complement. Poststreptococ-cal glomerulonephritis is characterized by proteinuria, hematuria, and red blood cell casts in urine. Serum sickness occurs 1-2 weeks after administration of antiserum, which acts as an antigen. Formation of circulating immune complexes is followed by deposition in blood vessels, joints, kidney, and heart. This results in an inflammatory reaction with necrosis, edema, vasodilatation, microthrombi, and ischemia.

CLINICAL CORRELATE

Horse antiserum will almost invariably cause serum sickness. It is much less frequent if human antiserum is used.

5. **Pathology**
   a. There is acute necrotizing vasculitis with fibrinoid necroses and neutrophilic exudation in arterial walls.
   b. Glomeruli in the kidney swell, and there is proliferation of endothelial and mesangial cells with a neutrophilic inflammatory exudate due to activation of the complement cascade.
   c. Immunofluorescence shows lumpy deposits along the basement membrane of blood vessels and glomeruli.
   d. Endocarditis with sterile vegetations, edema, and congestion of valves may also be seen.

CLINICAL CORRELATE

The major complement proteins that contribute to the glomerular lesions are C3b, which opsonizes, and C5a, which is chemotactic for PMNs.

6. **Therapy for immune complex-mediated reactions**
   a. Aspirin, antihistamines, and steroids to reduce inflammation
   b. Suppression of immune response by means of corticosteroids and cytotoxic immunosupppressive drugs (e.g., cyclophosphamide)
   c. Removal of immune complexes via plasmapheresis

D. **Type IV hypersensitivity, or delayed-type hypersensitivity (DTH).** Unlike the other types of hypersensitivity responses, which are mediated by antibody, DTH is **mediated by T cells** that have been sensitized to a particular antigen. An example of DTH is seen in **tuberculin skin testing** (TB tests), which measure previous T-cell exposure to the TB organism. **Contact hypersensitivity,** such as the response to poison ivy, is another characteristic type IV response.

1. **Delayed hypersensitivity reactions** are initiated by sensitized T_{DTH} cells. The reactions manifest as inflammation at the site of antigen exposure, which usually peaks 48-72 hours after exposure.
   a. Pathogenic mechanisms. Tissue damage results from the interaction between sensitized T cells and specific antigen, which leads to the release of lymphokines, direct cytotoxicity, or both.

CLINICAL CORRELATE

Delayed hypersensitivity responses are very important in the body's control of various chronic pathogens such as the mycobacteria and most fungi. Cytotoxic T cells are important in antiviral and antitumor responses.

b. Induction of delayed hypersensitivity requires that the T cells recognize immunogenic epitopes as well as determinants in major histocompatibility complex (MHC) gene products (MHC restriction). Cytokine products of helper T cells aid in regulating the response.
   (1) Helper T cells secrete IL-2, IFN-gamma, and lymphotoxin and help inflammatory reactions
   (2) IL-2 from the antigen-presenting cell also enhances the development of delayed hypersensitivities.

c. Expression of delayed hypersensitivity
   (1) On contact with epitope, activated T_{DTH} cells secrete lymphokines.
   (2) These products activate **macrophages** and recruit the immigration of more cells into the area.
   (3) The macrophages secrete a variety of biologically active compounds, including interleukins 1 and 6, tumor necrosis factor alpha, reactive oxygen metabolites, proteases, and other lysosomal enzymes.

2. **Contact hypersensitivity** is characterized clinically by eczema on the skin at the site of contact with allergen. It is induced by compounds (such as poison ivy catechols) that cross the skin and become conjugated to normal proteins. This conjugate sensitizes the person. T cells are able to recognize the conjugate after presentation by the Langerhans' cell.

3. **Tuberculin-type hypersensitivity** detects the prior exposure or sensitization of T cells to *Mycobacterium tuberculosis.* A protein extract (ppd) of the organism is injected under the skin. After 24 hours, the site of antigen exposure is infiltrated with lymphocytes and monocytes. At 48 hours, there is a more extensive infiltrate of mononuclear cells. The reaction is read by measuring the **degree of induration;** 12-14 mm is usually considered positive.

4. **Granulomatous hypersensitivity** is caused by the persistence of antigen (usually a microorganism) within macrophages. Microbial agents causing this type of reaction include *M. tuberculosis* and *M. leprae.* Characteristic target cells include epithelioid cells (which are derived from macrophages) and multinucleate giant cells. These reactions persist in the body for months or years.

5. **Expression of T-cell cytotoxicity** may also be linked to delayed hypersensitivity.
   a. Cell-mediated immunity and disease
      (1) T_{DTH} cells recognize the pathogen's antigens and secrete interferon gamma and other lymphokines, thereby activating macrophages to cytotoxic activity and helping B-cell responses.

IN A NUTSHELL

Hypersensitivity:

Type I
- *Anaphylaxis*
- *Atopy*
- *Requires previous exposure that sensitizes mast cells and basophils*
- *Mediated by IgE bound to mast cells or basophils that degranulate and release vasoactive mediators and chemotactic factors*
- *Examples: asthma, atopic dermatitis, eczema, and allergic rhinitis (hay fever)*

Type II
- *Cytotoxicity*
- *Antibody reacts with cell-surface epitopes*
- *Complement-mediated lysis or phagocytosis*
- *Examples: transfusion reactions, hemolytic anemia, and Goodpasture's syndrome*

Type III
- *Immune-complex mediated*
- *Activates complement*
- *Examples: serum sickness, arthus reaction, rheumatoid arthritis, and lupus nephritis*

Type IV
- *Delayed-type (DTH)*
- *Only type that is cell mediated (T cells)*
- *T cells (T_{DTH}) react with antigen in association with MHC class II → lymphokines released*
- *Examples: tuberculin skin testing, contact dermatitis, transplant and graft rejection*

(2) Cytotoxic T lymphocytes (Tc) recognize foreign antigens on most cells of the body and confer resistance by two mechanisms.
   (a) Secretion of interferon gamma that induces the production of antiviral proteins in cells of the body
   (b) Destruction of the cell via release of perforin with subsequent formation of transmembrane channels, Ca^{2+} ion influx, endonuclease activation, etc.
   (c) As a result, delayed hypersensitivity reactions provide resistance to chronic intracellular bacterial infections (e.g., tuberculosis), fungal, viral, and protozoan infections, and malignancies.

(3) As another expression of cell-mediated immunity, delayed hypersensitivity also plays a role in the rejection of grafted tissues and organs. (Humoral immunity also may be involved in allograft rejection.)

(4) Finally, delayed hypersensitivity reactions provide the basic mechanism of tissue injury in a variety of diseases, such as contact dermatitis and certain autoimmune diseases.

6. **Hypersensitivity pneumonitis** (also called extrinsic allergic alveolitis) refers to the lung parenchymal reaction to repeated inhalation of particulate allergens.
   a. **Pathogenesis.** Patients often have antibodies to the allergens, with deposition of antigen-antibody complexes in target (lung) tissue during the early phase of the disease. Recent evidence suggests that, in addition to immune complexes, delayed hypersensitivity may also play a role in hypersensitivity pneumonitis, particularly in the chronic disease process.
   b. Symptoms include fever, chills, chest pain, cough, and dyspnea, which occur 4-8 hours after exposure. In severe, chronic cases, irreversible lung damage (fibrosis) may occur.
7. **Therapy** for delayed hypersensitivity reactions includes:
   a. Drugs
      (1) Aspirin
      (2) Nonsteroidal anti-inflammatory agents
      (3) Corticosteroids
   b. Allergen avoidance

AUTOIMMUNE DISEASES

A defect in the central mechanism underlying self-recognition (autotolerance, selftolerance) can result in autoimmune responses. These are immune responses to antigens present in the host's own tissue and can be mediated by humoral (circulating antibodies, immune complexes) or cellular (delayed hypersensitivity) mechanisms.

A. **Several theories** have been proposed to explain autoimmune responses. It may represent an immunologic imbalance arising from the following:

1. **An excess of helper T (Th) cell activity** can be produced by coupling of chemicals, drugs (e.g., hydralazine), or viruses to self-antigen. The modified antigens would then recruit a reactive Th cell and elicit autoantibody production.
2. **Epstein-Barr virus** or **polyclonal B-cell activation** with materials such as bacterial lipopolysaccharide may result in autoantibody formation.
3. **An inability to down-regulate the immune response to self antigen.**
4. **Release of sequestered antigen** (e.g., from lens of the eye, sperm) not ordinarily available for recognition by the immune system. Release might occur via such means as trauma or infection.
5. **Molecular mimicry** in which a microbial antigen contains epitopes that are also found in self antigens. The host responds to the infectious agent (e.g., M protein of *Streptococcus pyogenes*) and the resultant immune response damages autologous tissue (e.g., myosin of cardiac tissues).
6. **Inappropriate expression of class II MHC molecules**. Pancreatic cells of IDDM patients have high levels of these molecules compared to healthy controls, where the antigen is practically nonexistent.
7. **Preferential activation of Th1 cells**.

B. **Genetic predisposition**

1. There is clearly a role for genetic factors in autoimmune disease. It is thought to be associated with those major histocompatibility complex genes that code for the class II antigens that are so important in presentation of antigens..
2. Autoimmune diseases tend to occur at a higher frequency in females. For example, the incidence of SLE in women is four to six times that in men, and rheumatoid arthritis is three to four times more common in females.

CLINICAL CORRELATE

Several autoimmune diseases seem to be linked to viral infections, particularly nervous system maladies.

- *Measles = multiple sclerosis, subacute sclerosing panencephalitis*
- *Rubella = insulin-dependent diabetes*
- *Influenza = Guillain Barré*

C. The **frequency** of autoimmune diseases increases with age. Mos appear in the 20-40 year age group.

D. **General signs of autoimmune disease** that may be of diagnosti importance include:

1. Elevated serum gamma globulin levels
2. Various autoantibodies
3. Depressed levels of serum complement
4. Immune complexes in serum
5. Lesions detected on biopsy (e.g., glomerular lesions resultin from deposition of immune complexes)

E. **Diagnostic tests** are designed to detect antibodies specific to th particular antigen involved in the disease. Certain facts should be pointed out, however.

1. Autoantibodies are not unique to autoimmune disease; for example, antinuclear antibodies may be found in tuberculosis, histoplasmosis, malignant lymphoma, and other neoplasms.
2. Patients with autoimmune disease may have more than one autoantibody and, if fact, may suffer from multiple autoimmune diseases.
3. While SLE is associated with antinuclear antibodies and rheumatoid arthritis is associated with rheumatoid factor, both antibodies may be found in both diseases. Patients with SLE may have 10-15 different autoantibodies.

F. **Therapy for autoimmune disease** involves several approaches.

1. In certain organ-specific diseases metabolic control may suf fice.
   a. In thyrotoxicosis (Graves' disease), antithyroid drugs such as propylthiouracil or methimazole may be prescribed. Surgical or radionuclide (^{131}I) ablation of the gland is also effective.
   b. Vitamin B_{12} is given to patients with pernicious anemia.
2. Agents such as **nonsteroidal anti-inflammatory drugs (e.g.** aspirin, indomethacin), **steroidal anti-inflammatory drug** (e.g., cortisone, which may also be immunosuppressive), an **immunosuppressive cytotoxic drugs** (e.g., cyclophosphamid azathioprine) are useful in treating disease symptoms.
3. **Anticholinesterase** drugs and **thymectomy** are of value myasthenia gravis.
4. **Plasmapheresis,** or plasma exchange therapy, appears to be value in certain diseases (e.g., Guillain-Barré, systemic lup

CLINICAL CORRELATE

Drugs are occasionally cited as precipitators of autoimmunity.

- *SLE may follow treatment with procainamide or hydralazine.*
- *D-penicillamine has been reported to induce myasthenia gravis, pemphigus, Goodpasture's disease, and lupus.*
- *L-dopa and α-methyl dopa are associated with hemolytic anemias.*

erythematosus, and Goodpasture's syndrome) by removing offending antibodies and immune complexes.

5. **Splenectomy** is of value in hemolytic diseases and idiopathic thrombocytopenic purpura.

G. **Systemic lupus erythematosus (SLE)** is a collagen vascular or connective tissue disease with a chronic remitting and relapsing course and multisystem pathology.

1. **Incidence** is about 1 in 1,000 with a female:male ratio of 9:1. It is more common in African Americans.
2. **Clinical diagnosis** is established by the criteria of the American Rheumatism Association. There should be four of the following signs or symptoms during the period of observation:
   a. Facial erythema (malar "butterfly" rash)
   b. Discoid lupus
   c. Raynaud's phenomena
   d. Alopecia
   e. Photosensitivity
   f. Oral or nasopharyngeal ulceration
   g. Arthritis without deformity
   h. LE cells
   i. Chronic false-positive serologic test for syphilis for more than 6 months
   j. Profuse proteinuria (> 3.5 g/day)
   k. Urinary cellular casts
   l. Pleuritis or pericarditis
   m. Psychosis or seizures
   n. One or more of the following: hemolytic anemia, leukopenia, or thrombocytopenia
3. **Clinical features.** SLE usually presents in the second or third decade. The onset may be insidious or acute. Most common presentations include fever of unknown origin, **arthritis, rashes,** and **renal involvement.** The course is highly variable with spontaneous exacerbations and remission.
   a. **Treatment** consists of corticosteroids, and immunosuppressive drugs.
   b. Mortality is most often from renal failure or infections.
4. **Pathology** is predominantly due to deposition of DNA-anti-DNA complexes, but may also result from direct autoantibody cytotoxicity in any organ.
   a. **Blood vessels** show acute necrotizing vasculitis in most affected tissues. Necrosis and fibrinoid deposits containing immunoglobulins, DNA, C3, and fibrinogen are seen, leading to a narrowed lumen.

CLINICAL CORRELATE

Anti-Ro antibodies are associated with congenital heart block seen in infants born to lupus mothers. These same infants may also have a transient annular erythematous skin rash. Anti-Ro antibodies are also found in high incidence in patients with Sjögren's syndrome.

b. **Joints** are involved in 80%-90% of cases. There is swelling and a mononuclear cell infiltrate of synovial membranes. Cartilaginous damage is rare.

c. **Skin** is involved in 80% of cases. The classical lesion is a maculopapular, erythematous rash over the bridge of the nose and malar eminences **(butterfly rash).** Microscopically, one sees liquefaction necrosis in the basal layer of the epidermis with immunoglobulin and complement deposition along the dermoepidermal junction.

d. **Kidney** involvement can be demonstrated in 70% of patients by light microscopy and almost 100% of cases using the electron microscope or immunofluorescent techniques.

e. The **heart** is involved in 50% of cases. **Libman-Sacks endocarditis** consists of bland verrucous vegetations of valves composed of fibrinoid material, necrotic debris, and inflammatory cells.

f. **Serosal membranes** are involved in 40% of cases. The pericardium and pleura are involved with effusions, fibrinous exudates, or, eventually, fibrosis.

g. **Lymph nodes** are involved in 60% of cases. They show enlargement, germinal center hyperplasia, and focal fibrinoid necrosis in vessels.

h. In the **CNS** (30% of cases), there is vasculopathy, precipitating infarcts, and hemorrhages, primarily involving small vessels.

i. In the **liver** (30% of cases), acute vasculitis with inflammatory infiltrates in the portal tracts is seen.

j. The **spleen** (20% of cases) may show capsular fibrosis, onion skin lesions, and concentric perivascular fibrosis.

k. In the **lungs** (10% of cases), interstitial pneumonitis and fibrosing alveolitis are seen.

H. **Scleroderma** is characterized by fibrosis throughout the body. It most often affects the skin, but also involves the kidney, lungs, striated muscle, heart, and gastrointestinal tract. There is a 3:1 female:male incidence ratio.

1. **Clinical features.** Scleroderma usually presents in the third to fifth decades with edema and thickening of the skin of the hands or Raynaud's phenomena. Also, arthralgias, dysphagia, respiratory distress, cor pulmonale, intestinal obstruction, hypertension, and renal failure may occur.

2. **Pathology**

a. Damage to **small blood vessels** may be prominent, causing ischemic injury and scarring. As in SLE, damage may be mediated by immune complex deposition.

NOTE

Immune complexes seen in the lupus kidney glomerulus include:

- *IgG or IgM*
- *Complement proteins*
- *DNA*
- *Fibrinogen*

IN A NUTSHELL

Systemic lupus erythematosus:

- *Young women*
- *Malar rash, joint pain, nephritis, endocarditis, pleuritis, pericarditis*
- *Positive ANA*
- *Anti-ds-DNA antibodies*
- *Anti-Sm antibodies*
- *Treated with steroids and immunosuppressive drugs*

b. **Skin changes** begin distally in the upper extremities and progress proximally, eventually involving all of the arms, chest, neck, and face. Lesions consist of increased dermal collagen, epidermal atrophy, and loss of skin appendages, which result in a limitation of movement, ulcerations, and a "drawn mask" appearance of the face.
c. In the **gastrointestinal tract,** fibrosis and atrophy of the esophagus are prominent, with ulceration of the mucosa. Scleroderma occasionally involves the stomach and causes malabsorption in the small intestine.
d. **Striated muscle** shows mixed inflammatory infiltrates and fibrosis.
e. **Joints** show sclerosis of articular collagen and periarticular connective tissue.
f. In the **kidney,** intimal proliferation and hyalinization of small arteries occur in two-thirds of patients.
g. The **lungs** show diffuse interstitial fibrosis.
h. The **heart** shows perivascular interstitial fibrosis.

3. **The CREST syndrome** (aka mild scleroderma) is a rather common collection of lesions characterized by **calcinosis, Raynaud's phenomena, esophageal dysfunction, sclerodactyly (scleroderma of the digits),** and **telangiectasia.**

CLINICAL CORRELATE

Anticentromere antibodies are pathognomonic for CREST syndrome.

I. **Sjögren's syndrome** is characterized by **keratoconjunctivitis sicca (dry eyes), chronic arthritis,** and **xerostomia (dry mouth).** It results from immunologic destruction of salivary and lacrimal gland duct epithelium. Sjögren's syndrome may be a primary disorder (sicca complex) or associated with another autoimmune disorder, such as SLE.

1. **Pathogenesis.** Sjögren's syndrome results from a lymphocytic infiltrate (predominantly helper T cells) and fibrosis of the ducts of lacrimal and salivary glands. It is associated with numerous autoantibodies. The primary form is associated with HLA-DR3.
2. **Clinical features.** Middle-aged women are most commonly affected.
   a. One-third of patients have the primary form; two-thirds are associated with another autoimmune disease such as SLE or rheumatoid arthritis.
   b. Patients present with blurred vision, a foreign body sensation in the eyes, cracks and fissures of the mouth, and dry mouth. The parotid gland is frequently enlarged. Vasculitis, Raynaud's phenomenon, hyperviscosity syndrome, and peripheral neuropathy are also associated with Sjögren's syndrome.

c. The term **"pseudolymphoma"** is used to describe enlargement and a pleomorphic infiltrate of lymph nodes. In this syndrome, there is a forty times greater chance of developing B-cell lymphomas.

3. **Pathology.** In Sjögren's syndrome, there is a destruction of glandular structure by lymphocytic and plasma cell infiltrates. Eyes exhibit inflammation and ulceration of the corneal epithelium. The oral mucosa exhibits atrophy, inflammation, and ulceration. The process occasionally involves respiratory passages, the esophagus (forming webs), and the stomach (atrophic gastritis).

AMYLOIDOSIS

Amyloidosis is characterized by the **deposition of amyloid protein in** the extracellular space of various organs and tissues. Generally, immunological mechanisms are thought to underlie the accumulation of amyloid, although the specific causes are diverse.

A. **Pathology**

1. With ordinary stains, amyloid appears as an eosinophilic, amorphous substance in extracellular spaces.
2. Under polarized light, amyloid displays **"apple-green" birefringence** when stained with Congo red.
3. Electron microscopy shows single fibrils 7.5-10 nm in diameter. X-ray crystallography reveals that the protein is arranged **in a β-pleated sheet.** Two major types of amyloid fibrils are found.

B. **Types of disease.** Amyloidosis may be localized, affecting particular organs or tissues, or systemic, affecting the entire body:

C. **Clinical manifestations**

1. Renal involvement causes proteinuria, cellular casts, and eventual renal failure.
2. Liver or spleen may be involved, with symptoms depending on the degree of infiltration.
3. Cardiac amyloidosis can produce arrythmias, cardiomyopathy and heart failure.
4. The prognosis is poor in generalized amyloidosis (mean survival 1-3 years). Treatment is often unsatisfactory.

TRANSPLANTATION AND TUMOR IMMUNOLOGY

A. **Transplantation immunology** deals with immune response to grafted tissues. The grafts may be from one part of the body to another site (e.g., **autografts,** as in mandible reconstruction, where a portion of the iliac crest is used to fashion a new jaw), or they may be from another person (e.g., **allograft or homograft,** where an organ such as a kidney, heart, or lung is removed from a donor and placed in a recipient whose organ(s) are failing).

1. Autograph grafts are accepted as "self" and do not induce an immune response.
2. Allografts are composed of foreign tissues and the recipient will respond immunologically; the relative immunogenicity varieties between organs.
3. Some grafts may involve synthetic material, as occurs in heart valve replacement and artificial heart implantation surgery. Only rarely are tissues and/or organs transplanted across species barriers. These grafts, termed **xenografts,** are usually rejected very rapidly and are not intended to be permanent organ replacements.
4. Grafting of normal tissue between identical twins or from one animal in an inbred line to another animal in that same line succeeds as if there were no immune barriers. These are **isografts,** and the tissues bear a **syngeneic** relationship. However, transplantation between genetically nonidentical animals will result in an immune rejection phenomenon, which may or may not be prevented or aborted by the use of immunosuppressive drugs. Rejection is due to the recipient's recognition of, and immunologic response to, glycoprotein histocompatibility antigens in the membranes of cells in the grafted tissue. Certain antigens of the red cell system may also act as transplantation antigens. One cannot violate incompatibility in the red cell system and expect graft survival.
5. **The donor-recipient workup.** The compatibility of donor and recipient must be maximized.
   a. ABO blood group compatibility is first established.
   b. Class I and Class II HLA tissue typing is performed.
   c. Cross-matching is used to test for recipient performed antibodies against the donors HLA class I and class II antigens.
   d. Other cellular assays such as the mixed lymphocyte reaction (MLR) may be used to determine if the donor cells stimulate blastogenesis in the recipient's lymphocytes.

CLINICAL CORRELATE

Larger organs such as liver and heart/lung transplants are less immunogenic than skin and kidney transplants. They may also survive better because of the vast amount of antigens they contain (refer back to the "Tolerance" section of Basic Immunology).

6. **Histocompatibility gene complex**
   a. **HLA** is an acronym for human leukocyte antigens, so termed because lymphoid cell membranes are particularly rich in these substances. The genes that dictate the immunologic specificity of these antigens occur in allelic form; they are polymorphic, e.g., multiple genes may occur at a single locus but only one will be expressed per locus (per chromosome). In addition they are codominant in expression, which means that, if an individual is heterozygous, both antigens will be present on the cells.
   b. **Classification of HLA molecules**
      (1) **Class I antigens:** The HLA-A, -B and -C antigens are found on all nucleated human cells. Structurally, the class I antigens are composed of a glycoprotein in non-covalent association with a nonpolymorphic beta-2-microglobulin. Class I molecules present endogenous antigens and are also involved in MHC restriction of cell-mediated cytolysis.
      (2) **Class II antigens:** HLA-D antigens (D=DP, DQ, and DR) are found chiefly on the surfaces of antigen-presenting cells, including macrophages/monocytes, resting T lymphocytes (in low amounts), activated T lymphocytes, and B lymphocytes. The HLA-D region antigens have been shown to be involved in antigen presentation by macrophages to T lymphocytes as well as in efficient collaboration between B and T cells.

7. **Transplant rejection**
   a. In graft rejection, an allogenic peptide is expressed in the groove of an allogenic MHC I molecule. This combination stimulates the host in much the same way as a viral peptide. **T-cell-mediated rejection** results in acute rejection 10-14 days after a graft is placed. It is mediated by both delayed-type hypersensitivity and T-cell cytotoxicity and requires helper cells and cytotoxic T cells.
   b. **Antibody-mediated rejection** may be caused either by pre-formed (hyperacute) or subsequently formed antibodies (late acute rejection vasculitis). Hyperacute rejection may be mediated either by ABO blood group antibodies (usually IgM) or anti-HLA antibodies (usually IgG) from a previous transplant, or, in the case of multiparous women, from immunization during pregnancy.

8. **Methods to minimize rejection**
   a. Matching for at least 4 of the HLA antigens reduces the incidence of rejection.

b. **Immunosuppression** of the graft recipient with monoclonal antibodies vs. helper T cells, azathioprine, steroids, or cyclosporin A kills lymphocytes or reduces IL-2 secretion.
c. Antilymphocyte serum decreases the level of lymphocytes, thereby prolonging graft survival.
d. With the use of high doses of immunosuppressive drugs, one often gets into trouble with infection and/or malignancy. About 25% of the deaths that occur in kidney transplants are due to sepsis.

9. **Pathology of rejection**
   a. **Hyperacute rejection** shows arteritis, thrombosis, and ischemic necrosis as a result of an Arthus-like reaction. The graft does not become vascularized and appears dusky and mottled within seconds to minutes.
   b. **Acute rejection** has two components. The humoral component causes vasculitis; the cellular component causes death of tissue cells of the graft with accompanying decrease in function. It occurs weeks after transplantation.
   c. **Chronic rejection** shows intimal fibrosis, causing parenchymal ischemia, atrophy, and fibrosis. Its time course is months to years.
   d. **Recurrence of the original disease** may also be seen in diseases such as diabetes, glomerulonephritis, or pyelonephritis; this will obviously compromise graft function.
10. **Graft-versus-host disease (GVH)** results from transplantation of immunologically active cells into an immunocompromised recipient, resulting in a "rejection" reaction of these graft cells against host tissues. It most often occurs after **bone marrow transplantation.** Newer technology is able to remove immunologically active donor cells and minimize GVH. Cyclosporin A has been used successfully to control these adverse transplant events.

B. Tumor immunology

1. **Tumor antigens. Tumor-associated antigens (TAA)** are those antigens present on tumor cells but that may also be found on some normal tissues. TSA refers to **tumor-specific antigens.** These antigens are found only on tumor cells and not on normal tissues. **Tumor-specific transplantation antigens (TSTA)** are a specialized form of TSA; they are the antigens to which the immune system must respond if it is to eliminate the growth of the tumor *in vivo.*
   a. **Antigens of chemically induced tumors.** When tumors are induced in experimental animals using a chemical carcinogen, the TSTA of each individual tumor will be unique to

CLINICAL CORRELATE

Hyperacute rejection is due to preformed antibody against the graft donor, as may occur in multiparous women.

that tumor. Tumor antigens of chemically induced tumors are mutational events that occur as a result of the chemical carcinogen that is also a mutagen. The mutation is random and would not be found in another tumor unless millions were compared. The tumor antigen is a mutated normal cellular protein.

b. **Antigens of virally induced tumors.** The TSTA of tumors that arise as a result of oncogenic viruses are completely cross-reactive. The genetic information for the TSTA induced by viral agents comes from the virus. The virus enters the cell and integrates its genome into the host cell DNA. Proteins derived from this genetic material are also processed by the endogenous route and expressed on the surface of the cell in association with MHC class I molecules. When a different virus is used for induction of the tumor, then different proteins are made and the TSTA is also different.

c. **Oncofetal antigens.** Other types of antigens that may be found on tumor cells are called oncofetal antigens. These antigens are normally present on tissues during fetal development but their synthesis is repressed before birth. When cells undergo malignant transformation, these genes are derepressed and the proteins are once again synthesized. In human medicine, oncofetal antigens have been used as markers for the growth of some tumors.

(1) **Alpha fetoprotein (AFP)** is a serum protein secreted by the fetal liver. It is increased in most patients who have **hepatomas** but it is also increased in other types of cancer. Originally, it was thought that AFP levels might be useful in the diagnosis of these malignant diseases; however the protein is also elevated in a variety of non-malignant disorders of the liver. While diagnosis of malignancy by AFP levels is not possible, AFP levels have been used to establish prognosis and monitor the success of therapy for tumors that secrete this protein.

(2) **Carcinoembryonic antigen (CEA)** is another human oncofetal antigen that is elevated in patients with **carcinoma of the colon** or pancreas. CEA levels are present in normal individuals at low levels because it is normally present in the gut, liver, and pancreas. Elevated CEA levels occur with other cancers including lung, breast, and prostate. As with AFP, CEA levels can also be elevated

NOTE

There are several known oncogenic viruses:

- *Human papilloma virus → Cervical and penile cancer*
- *Epstein-Barr virus → Burkitt's lymphoma, nasopharyngeal cancer*
- *Hepatitis B and C viruses → Hepatocellular carcinoma*
- *HTLV 1 → Adult T cell leukemia*

some nonmalignant disorders. Fifteen percent of heavy smokers will have elevated CEA levels. While not useful for diagnosis of disease, elevated CEA levels can contribute to diagnosis, provide information regarding prognosis, and be used to monitor the success of therapeutic intervention for carcinoma of the colon.

(3) **Leukemia antigens.** Specific antigens are associated with certain leukemias, which makes blood tests for these antigens useful in diagnosis.

(a) **Terminal deoxynucleotidyl transferase (TdT)** is an enzyme marker for immature cells of the hematopoietic system. It is found in patients with **leukemia and lymphomas.**

(b) **Common acute lymphoblastic leukemia antigen (CALLA),** also known as CD10 antigen, is found on the surface of tumor cells in 80% of patients with acute B-cell lymphoblastic leukemia (ALL) and in 45% of patients with chronic myelogenous leukemia (CML). It is also found on immature B cells.

Antigen	Cancer
Alpha fetoprotein (AFP)	Liver, testes
Carcinoembryonic (CEA)	Colon
Chorionic gonadotropin	Trophoblastic
Immunoglobulin	Myeloma
Prostate-specific antigen (PSA)	Prostate
CALLA	B-cell leukemia

Table 2-1. Useful tumor markers.

2. **Immune surveillance** refers to the role of the immune system in preventing cancer in immunologically normal individuals. It is believed that one of the roles of the immune system is to regularly eliminate cells of the body that may undergo malignant transformation. The fact that immunodeficient patients have an increased incidence of tumors has been used to support this theory. Current thinking suggests that this function may be carried out by natural killer cells.

3. **Mechanisms for rejection of tumors**

   a. **Antibody and complement.** In many experimental and human situations, antibodies specific to tumor cells can be found in the serum of the tumor-bearing host. However, for the most part, experimental evaluation has shown that antibody is not very effective at eradicating tumor cells *in vivo.*

   b. **Cytotoxic T cells (Tc).** $CD8^+$ cytotoxic T cells are very potent killers of tumor cells. These cells recognize foreign peptides

NOTE

Oncofetal antigens are found in fetal tissues and in malignant tissues. However, their production is also seen in nonmalignant conditions, hence they are not diagnostically useful. Their value is in prognosis/monitoring.

CLINICAL CORRELATE

Natural killer cells are responsible for elimination of precancerous cells from the body (immune surveillance). Their numbers are sometimes increased via in vitro manipulations so that they can be used therapeutically.

on tumor cells and release a variety of molecules that c
kill the tumor target. One of these molecules is **perfo**
which polymerizes in a manner similar to the membra
attack complex of the complement system and punch
holes in the membrane of the tumor. Not only does t
provide a mechanism whereby the target might lyse, b
enzymes released by the Tc cell and that are toxic to t
tumor cell can enter through these holes. These enzym
(e.g., proteases and nucleases) are called **granzymes. TNF**
and IFN-γ are also produced by the Tc cell. These cytokin
interact with their respective receptors on the membra
of the tumor cell and are able to promote killing of t
tumor.

c. **Helper T cells.** Although tumor antigens are expressed
conjunction with class I MHC, there is evidence that Class
MHC expression also occurs. This may be due to engulf
ment of dying tumor cells by macrophages and expression
of peptides from these molecules via the exogenous anti
gen processing route. Helper T cells of the Th1 type ar
very active in augmenting nonspecific mechanisms of
tumor rejection. **Lymphotoxin** (also called TNF-β) produced
by the Th1 cell can kill tumor cells directly. Additionally,
IFN-γ released by the Th1 cell activates NK cells to exhibit
enhanced cytotoxicity toward tumor targets. Interferon
also activates macrophages to release molecules that are
cytotoxic to tumor cells (e.g., reactive oxygen and nitrogen
intermediates).

d. **Natural killer cells.** NK cells are cytotoxic to tumor cells;
they secrete a variety of molecules that attack the tumor
target. **Natural killer cell cytotoxic factor (NKCF)** is active in
this regard. Additionally, these cells secrete granzymes and
perforin. NK cells also release IFN-γ and TNF. NK cells
include TNF-α, TNF-β and IFN. NK cells have the ability to
recognize malignant cells and kill them, but they are not
cytotoxic for normal cells. Receptors (probably several) are
responsible for this interaction.

e. **Activated macrophages.** Normally, macrophages are not
cytotoxic in their resting state. However, after they come in
contact with IFN-γ they become activated and are highly
cytotoxic to tumor cells. Like NK cells, activated
macrophages kill tumor cells but do not kill normal cells.
They release cytotoxic factors, including TNF-α, reactive
oxygen intermediates (ROI) such as H_2O_2 and superoxide
anion, as well as the reactive nitrogen intermediate (RNI)
nitric oxide. All of these molecules are toxic to tumor cells.

f. **Antibody-dependent cell-mediated cytotoxicity (ADCC)** is a mechanism that helps cytotoxic cells come in better contact with tumor targets. Because NK cells and macrophages have Fc receptors, antibodies can associate with these cells via the Fc portion of the antibody molecule. If the antibody is directed at a tumor cell's surface antigen, then the antibody can help the NK cell or the macrophage come in close contact with the tumor cell.

4. **Factors that favor tumor growth.** Even though the immune system has a wide variety of weapons to inhibit the growth of tumors, the fact remains that the large majority of cancer patients will die if they are not treated by surgery and/or chemotherapy. Tumor immunologists have discovered a variety of mechanisms that tumors use to subvert the immune system.
   a. **Tumor cell heterogeneity.** Individual tumor cells isolated from a single tumor are very heterogenous. As the tumor grows and differentiates, the cells change in many ways, including antigenic make-up. This property allows the tumor cell to escape from Tc cells directed at specific antigens.
   b. **Rapid growth.** Tumor cells grow much more rapidly than normal cells. The tumor cell may simply be able to outstrip the ability of the immune system to respond. Many tumors secrete growth-promoting factors (e.g., epidermal growth factor) that promote their own proliferation.
   c. **Release of immunosuppressive factors.** Many tumors release factors that are immunosuppressive. Included among these are alpha fetoprotein and the cytokine TGF-β.
   d. **Shedding of antigens.** Tumors cells have been shown to shed their antigens to a much greater degree than normal tissues. This large amount of soluble antigen can be immunosuppressive via the induction of immunologic anergy or through blocking of cytotoxic T-cell function (discussed below).
   e. **Serum-blocking factors.** Factors have been found in the serum of tumor patients that are able to block the cytotoxic activity of the Tc cell. These factors are present in the serum when a large tumor is present and are lost from the serum soon after surgical removal of the tumor. Tumor antigen, antitumor antibody, and antigen-antibody complexes can all function as serum-blocking factors. However, antigen-antibody complexes are by far the most efficient in their inhibition of the cytotoxic T-cell response.

IN A NUTSHELL

Mechanisms of tumor rejection:

- *Antibodies*
- *Complement*
- *Cytotoxic cells*
  - *T cells: T_{DTH}, Tc*
  - *Macrophages*
  - *NK cells*
  - *ADCC cells*

IN A NUTSHELL

Factors that favor tumor growth:

- *Rapid growth rate*
- *Antigenic heterogeneity*
- *Synthesis of immunosuppressants*
- *Secretion of tumor antigens*
- *Induction of nonprotective, competing antibodies*

PART II

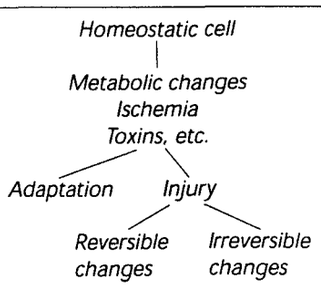
PATHOLOGY

Cellular injury and inflammation are basic processes underlying all cellular and tissue changes in health and disease. These disease states may result from neoplasms, genetic or metabolic disorders, or from the introduction of exogenous toxic materials. This chapter will focus on the underlying processes causing cellular disease states, as well as the mechanisms by which the cell adapts to its external stimuli.

CELLULAR INJURY AND ADAPTATION

athologic processes are manifested at the cellular, organ, and vhole body levels. Cell survival depends on the maintenance of iomeostasis, a stable internal environment, which requires a contant supply of metabolic energy and active transport processes. 'rior to the microscopic appearance of cell injury, critical alterations of basic biochemical pathways must occur. When this homeostatic tate is disrupted sublethally, the cell first adapts to the change. If he cell is unable to fully adapt, cell injury ensues. Injury at first caus-:s reversible changes but may progress ultimately to irreversible njury and cell death. The ability of the cell or organ to tolerate njury depends on the severity, duration, and type of insult, as well is the adaptive capacity of the tissue.

IN A NUTSHELL



A. Causes of cellular injury

1. **Hypoxia,** a lack of oxygen, leads to the inability of the cell to synthesize sufficient ATP. The loss of ATP production results in a failure of the membrane sodium pump, increased glycolysis, and progressive detachment of the ribosomes from the rough endoplasmic reticulum. Hypoxia can result from:
   a. Loss of blood supply (ischemia) due to decreased arterial flow.

   b. A decrease in the oxygen-carrying capacity of the blood due to anemia or carbon monoxide poisoning; CO produces a stable complex with hemoglobin, blocking O_2 transport.
   c. Poisoning of the enzymes of oxidative phosphorylation by toxins such as cyanide, rotenone, and antimycin A.
2. **Chemical injury** can lead to a disruption of the physical structure of the cell or to a breakdown of the biochemical processes of the cell. For example, chemicals can alter membrane permeability or block the action of an enzyme by binding either to the enzyme or to its cofactor.
3. **Physical injury,** such as crush injuries, gunshot wounds, burns, frostbite, radiation, and pressure changes, can lead to cell death and inflammation.
4. **Infections.** Virtually all aspects of cellular metabolism are affected by biologic agents infecting the cell.
   a. **Viruses** invade cells, commandeer synthetic machinery, and may release proteins that are toxic to host cells and cellular metabolism.
   b. **Bacteria** release exotoxins (e.g., phospholipases) or produce endotoxins (e.g., lipopolysaccharides) from their cell walls. Both cause cell injury and possibly death.
   c. Viruses, bacteria, parasites, and fungi can cause the host to initiate a cellular (e.g., macrophages, T cells) or humoral (e.g., IgG, IgM) immunologic reaction to the invader.
5. **Immunologic reactions.** Although the immune response is tightly regulated, it can result in injury as manifested by an anaphylactic reaction, or autoimmune diseases. Direct injury to an organism can result from the absence of an immune reaction.
6. **Genetic disorders,** which can present as biochemical abnormalities, can lead to the accumulation of toxic products or the inability to metabolize various compounds due to enzyme defects, such as cystic fibrosis, sickle cell anemia, and Tay-Sachs disease. Acquired genetic defects (mutations) in genes that govern cell growth and differentiation (oncogenes) may lead to the development of cancer.
7. **Nutritional or vitamin deficiencies,** hypervitaminosis, inadequate calorie intake, or inadequate protein intake may all lead to cellular atrophy or even death.
8. **Aging** can lead to the breakdown of normal cellular machinery, ultimately leading to death of the cell. Some cells, such as gut epithelium or bone marrow stem cells, continuously renew, while others, such as neurons and skeletal muscle, may age and die.

B. Cellular changes during injury

1. **Cloudy swelling** results from disruption of the integrity of the plasma membrane. By inhibiting oxidative phosphorylation, hypoxia results in decreased ATP production. The loss of ATP affects the ouabain-ATPase, causing a failure of the membrane Na^+ pump. Disruption of the cells' osmotic pumps leads to an influx of Ca^{2+} and water and an efflux of K^+. The cells swell and the endoplasmic reticulum becomes dilated. Although initially reversible, ultimately injury to the cell becomes irreversible if ATP is not restored.
2. **Membrane damage** plays a central role in the pathogenesis of irreversible injury. The membrane can be damaged from the loss of membrane phospholipids, breakdown of the cytoskeleton, production of toxic oxygen intermediates, and the production of lipid products, which by themselves can have a detergent-like effect on the plasma membrane.
3. **Dilation and swelling** of the endoplasmic reticulum lead to detachment of ribosomes, which leads to a decrease in protein synthesis.
4. **Mitochondrial swelling** results in an accumulation of Ca^{2+}, which uncouples oxidative phosphorylation.
5. **Lysosomes rupture,** releasing their digestive enzymes into autophagic vacuoles or into the cytosol.
6. **Nuclear changes** proceed from chromatin clumping to pyknosis with degeneration and condensation of nuclear chromatin. This can be followed by karyorrhexis (i.e., nuclear fragmentation) or karyolysis (i.e., dissolution of the nucleus).

C. Expected pathologic changes in cell death and injury

1. **Coagulative necrosis** is the most common form of necrosis in cells without large numbers of lysosomes. The cell is converted into a homogeneous, eosinophilic mass with loss of the nucleus but preservation of cellular shape. Coagulative necrosis typically occurs after sudden ischemia, thermal injury, or toxin injury. The heart is the most common example of an organ undergoing coagulative necrosis following an injury.
2. **Liquefaction necrosis** results from cellular destruction by hydrolytic enzymes involved in autolysis and heterolysis. Typically, liquefaction necrosis occurs in brain infarcts and pancreatic necrosis. Liquefaction by leukocytic enzymes is called **suppuration,** and the resultant fluid is called **pus.**
3. **Caseous necrosis** is a combination of coagulation and liquefaction necrosis, which produces tissue that is grossly soft, friable, and "cheese-like." Caseous necrosis is characteristic of tuber-

IN A NUTSHELL

Cellular changes during injury:

- *Cloudy swelling from cell membrane disruption*
- *Membrane damage*
- *Endoplasmic reticulum swelling*
- *Mitochondrial swelling*
- *Lysosomal swelling*
- *Nuclear changes*
- *Apoptosis*

NOTE

Squamous carcinomas often necrose in the center of invasive nodules due to their rapid growth.

culosis, some granulomas and fungal infections, and the center of certain malignancies.

4. **Enzymatic fat necrosis** is caused by the action of lipases on fatty tissue. It is characteristic of tissues adjacent to acute pancreatic necrosis.
5. **Gummatous necrosis** is seen in the late stage of syphilis; grossly, it differs from coagulative and liquefactive necrosis by its gelatinous appearance.
6. **Apoptosis** is a specialized form of programmed cell death that is characterized by:
   a. Chromatin condensation and formation of cytoplasmic membrane blebs (cell surface deformities caused by cytoskeletal disruption)
   b. Breakdown of DNA into nucleosome-sized fragments
   c. RNA and protein synthesis
   d. A minimal inflammatory response

D. **Other cellular alterations during injury**

1. **Intracellular accumulations**
   a. **Lipids**
      (1) Triglycerides (e.g., fatty change in liver cells)
      (2) Cholesterol (e.g., atherosclerosis)
      (3) Complex lipids (e.g., sphingolipid accumulation)
   b. **Proteins** (e.g., renal epithelial cells in proteinuria)
   c. **Glycogen and complex carbohydrates** (e.g., glycogen storage diseases, mucopolysaccharidoses)
   d. **Pigments** are colored substances, either normal cellular constituents or abnormal constituents that lead to deposits.
      (1) **Exogenous pigments.** Anthracotic pigmentation of the lung is secondary to inhalation of carbon dust.
      (2) **Endogenous pigments.** Lipofuscin (wear and tear pigment), melanin, hemosiderin, and bilirubin all may accumulate either in the cells that made them or in macrophages.
2. **Calcification**
   a. Dystrophic calcification appears in areas of necrosis due to precipitation of calcium phosphate in low pH.
   b. Metastatic calcification caused by hypercalcemia (malignancy, hyperparathyroidism) is due to precipitation of supersaturated solutions of calcium phosphate.

E. **Adaptive cellular responses to injury**

1. **Atrophy** is a loss of cells or cell substances, resulting in decrease in cell and organ size. The causes of atrophy are d... use, ischemia, aging, malnutrition, and lack of hormonal ...

neural stimulation. Atrophy of an organ may be due to loss of cells, a decrease in cell size, or both.

2. **Hypertrophy** is an increase in both cell and organ size. It is due to an increased mechanical demand, such as that seen in striated muscle of weight lifters or cardiac muscle in hypertension. It can also be seen with an increased endocrine stimulation. Hypertrophy can be physiologic or pathologic. Hypertrophy of an organ may be due to an increase in cell number (lactating breast), an increase in cell size (skeletal muscle), or both (many cancers).
3. **Hyperplasia** is an increase in the number of cells. It is often associated with hypertrophy. Some cell types are unable to exhibit hyperplasia (e.g., nerve, cardiac, skeletal muscle cells). It can be physiologic or pathologic. Physiologic causes include compensatory (e.g., after partial hepatectomy), hormonal stimulation (e.g., breast development at puberty), or antigenic stimulation (e.g., lymphoid hyperplasia).
4. **Metaplasia** is a reversible change of one cell type to another, usually in response to irritation. It has been suggested that the replacement cell is better able to tolerate the environmental stresses. For example, bronchoalveolar epithelium undergoes squamous metaplasia in response to chronic irritation of tobacco smoke.

NFLAMMATION AND REPAIR

nflammation enables the body to resist infection. Inflammation occurs in response to injury, which can result from hypoxia, chemicals, drugs, physical agents, microbial agents, immunologic reactions, nutritional imbalances, genetic defects, or aging. The acute inflammatory response occurs over seconds, minutes, hours, and days after the initial insult, while the chronic inflammatory response can continue for weeks, months, and even years after the primary injury.

A. Acute inflammation

1. **Cardinal signs of inflammation**
   a. Rubor (redness)
   b. Calor (heat)
   c. Tumor (swelling)
   d. Dolor (pain)
   e. Loss of function
2. **Pathophysiology.** The acute inflammatory response begins with changes in the vasculature. There is a transient vasoconstriction followed by vasodilatation of the affected area. Ultimately, the blood flow slows as the vasculature becomes

leaky. First, there is a **transudate** of comparatively protein-free fluid into the extravascular space, followed by an **exudate** of proteins, cells, and plasma, depending on the severity of the injury.

a. **Vascular changes**

(1) There is a transient vasoconstriction of arterioles followed by vasodilatation with opening of supplementary capillary beds, which leads to an increased blood flow. Vasodilatation can be mediated by histamine, bradykinin, and prostaglandins.

(2) Increased vascular permeability or vascular leakage is due to endothelial cell and pericyte contraction, transiently affecting venules; direct endothelial cell injury affecting all microvessels; leukocyte-dependent injury to vessels; and regenerating endothelium.

(3) Chemical mediators of increased vascular permeability include the vasoactive amines, histamine and serotonin, which are stored in the granules of mast cells, basophils, and platelets. They act exclusively on venules (not capillaries). The complement components C3a and C5a are both anaphylatoxins and cause the release of more vasoactive amines. Bradykinin, an end-product of the kinin cascade, can also cause pain. Leukotrienes (i.e., LTC_4, LTD_4, LTE_4) also individually produce increased vascular permeability.

(4) Slowing of the circulation, resulting from increased blood viscosity due to extravasation of fluid, allows leukocytes to marginate, roll, and then adhere to endothelium via specific receptors. These include the integrin family of glycoproteins (LFA-1) on the neutrophil and intercellular adhesion molecule (ICAM-1) on the endothelial cell. Adhesion is Ca^{2+}-dependent.

(5) Leukocytes emigrate from the vasculature. The marginated, adherent cells extend pseudopods between the endothelial cells. They then move between the endothelial cells, migrating through the basement membrane, towards the inflammatory stimulus.

(6) **Chemotaxis** is the attraction of cells towards a chemical mediator that is released in the area of inflammation. Important chemotactic factors include bacterial products, such as *N*-formylmethionine (a prokaryotic product), LTB_4, many factors liberated from leukocytes, and IL-8. The two most important chemotactic factors for neutrophils are C5a and IL-8.

(7) **Phagocytosis.** Neutrophils and macrophages engulf and destroy foreign material. The particle to be phago-

NOTE

Transudate *is a protein-free fluid leaked to the extravascular space;* ***exudate*** *is fluid containing proteins and cells leaked to the extravascular space. Transudate is usually due to pressure differences between the vasculature and intracellular space (e.g., pulmonary edema in CHF). Exudate is usually due to increased permeability of endothelial cell barriers and chemotactic factors attracting white blood cells (e.g., lung cancer, infection, toxins).*

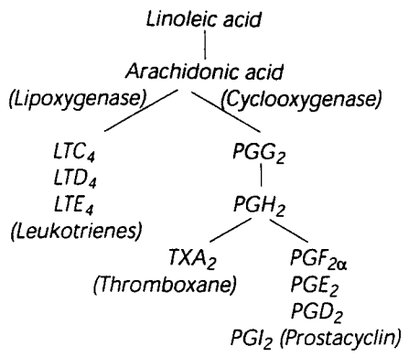
tosed can be coated with serum opsonins, such as IgG and C3b. These facilitate phagocytosis by allowing the particle to bind to complement receptor 1 (CR1) and the Fc receptor on the surface of the cell. After the particle is engulfed, the phagocytic vacuole fuses with a lysosome, forming a phagolysosome complex. The lysosome disgorges its contents into the fused vacuole. The offending particle (e.g., a bacterium) is then broken down via the action of reactive oxygen species, acid hydrolases, neutral proteases, and lysozyme.

b. **Chemical mediators of inflammation.** Metabolites of arachidonic acid metabolism mediate many of the important aspects of the inflammatory response. The processing of arachidonic acid occurs via two pathways:
   (1) **Cyclooxygenase pathway,** leading to prostaglandin formation; certain prostaglandins (i.e., PGI_2, PGD_2, PGE_2, $PGF_{2\alpha}$) mediate vasodilatation and pain.
   (2) **Lipoxygenase pathway**, leading to leukotriene synthesis; certain leukotrienes (i.e., LTB_4) are involved in chemotaxis and increasing vascular permeability.

c. **Actions of anti-inflammatory drugs**
   (1) Aspirin and the NSAIDs exert their anti-inflammatory effect by inhibiting prostaglandin synthesis (cyclooxygenase).
   (2) Corticosteroids most likely act by preventing the transformation of phospholipid into arachidonic acid by inhibiting the membrane enzyme phospholipases. Therefore, they inhibit both prostaglandin and leukotriene synthesis. Corticosteroids also impair leukocyte migration towards an inflammatory focus and stabilize lysosomal membranes.

3. **Chronic inflammation.** Acute inflammation can be resolved completely or progress to chronic inflammation. The activated monocyte-macrophage plays a central role in chronic inflammation. It secretes enzymes such as neutral proteases (i.e., elastase, collagenase) and acid hydrolases (i.e., phospholipases), which can digest connective tissue. The activated monocyte-macrophage acquires the capability in a few days of secreting various plasma proteins, such as complement components C1 to C5, reactive metabolites of oxygen, leukotrienes, prostaglandins, cytokines (i.e., IL-1, tumor necrosis factor), as well as various growth factors (i.e., fibroblast growth factor, epidermal growth factor, platelet-derived growth factor). Chronic inflammation occurs if the offending agent cannot be removed (e.g., nondegradable foreign bodies, parasites) or if the tissue is subjected to repeated episodes of acute inflammation, such as recurrent cholecystitis.

BRIDGE TO BIOCHEMISTRY



NOTE

The main cellular component in acute inflammation is the neutrophil; the main cellular component in chronic inflammation is the monocyte-macrophage.

1. **Composition of the cellular infiltrate**
   a. Cellular infiltrate is primarily mononuclear with proliferation and maturation of monocytes into macrophages (e.g., interferon-γ).
   b. Fibroblasts are recruited and proliferate; small vessels proliferate and subsequent collagen deposition results in fibrosis and scarring.
   c. Lymphocytes, plasma cells, and eosinophils are also present in sites of chronic inflammation.
   d. Neutrophils are occasionally continuously attracted in chronic inflammation associated with pus.
2. **Chronic granulomatous inflammation** occurs if a substance cannot be completely removed (e.g., asbestos, silica, tuberculous bacilli) or if a cell-mediated reaction is initiated against an agent. It is most frequently seen in tuberculosis (caseating granulomas), sarcoid (noncaseating granulomas), or with foreign bodies. Granulomas are also seen in Crohn's disease, gout, rheumatoid arthritis, and in fungal and parasitic infections.
   a. Granulomas are small (0.5-2 mm) and consist of aggregations of macrophages, which can be transformed into epithelioid cells with occasional multinucleated giant cells. They are often surrounded by lymphocytes as well as plasma cells and fibroblasts.
   b. Epithelioid cell-modified macrophages with abundant eosinophilic cytoplasm contain large amounts of endoplasmic reticulum, Golgi, and vesicles, which indicate a secretory rather than a digestive function.

C. **Repair.** Almost as soon as the inflammatory process begins, the repair of the damaged cells and tissues starts. Repair involves two separate processes: regeneration of the damaged tissue by cells of the same type and replacement by connective tissue. Together they constitute wound healing.

1. **Regeneration.** Different tissues have different regenerative capacities.
   a. **Labile cells** regenerate throughout life. This cell type includes surface epithelial cells, such as those lining the skin, oral cavity, vagina, cervix, hematopoietic, splenic, and lymphoid cells, and the mucosal cells of all excretory organs.
   b. **Stable cells** replicate at a low level throughout life but are dormant unless stimulated by some initiating event; these include the liver, pancreas, kidney, vascular endothelium and smooth muscle.

c. **Permanent cells** cannot replicate and include neurons, skeletal muscle, and cardiac muscle.

2. **Replacement** of a damaged area by connective tissue involves migration and proliferation of fibroblasts into the damaged area, deposition of extracellular matrix, formation of new blood vessels, and reorganization of the connective tissue into a scar. Macrophages are usually present initially as the area is being remodeled. Neutrophils, eosinophils, lymphocytes, and mast cells can also be present.

3. **Wound healing** involves collagen synthesis and degradation. Various growth factors, such as PDGF, transforming growth factors (TGF) α and β, FGF, and cytokines, such as tumor necrosis factor (TNF) and IL-1, stimulate collagen synthesis. Collagen can be broken down by various proteases such as collagenase, which can be secreted by macrophages and neutrophils migrating into the damaged area. Wound healing may be prolonged by foreign bodies, infection, ischemia, diabetes, malnutrition, or scurvy.

   a. **Primary union by first intention** occurs when there has been little surrounding tissue damage. The wound is clean and the wound edges are closely approximated.
      (1) The wound fills with clotted blood, forming a scab.
      (2) Neutrophils line the wound edge within 24 hours.
      (3) A thin, continuous epithelial cover appears within 24-48 hours.
      (4) Macrophages replace neutrophils, while granulation tissue fills in the wound; the epithelial covering thickens.
      (5) By day 5, collagen fibers laid down by fibroblasts cross the incision following fibrin and fibronectin matrices.
      (6) Collagen continues to be synthesized, and the scar becomes increasingly avascular.
      (7) Full maturation of a scar requires up to 1 year.

   b. **Secondary union by secondary intention** occurs when the two skin edges are not in contact. It requires larger amounts of granulation tissue to fill in the defect; it is characterized by significant wound contraction and is mediated by myofibroblasts.

CLINICAL CORRELATE

Scurvy is caused by vitamin C deficiency; since vitamin C is a necessary cofactor for the cross-linking of collagen, scurvy can result in impaired wound healing because the cross-linking of collagen is essential for its tensile strength.

CIRCULATORY DISTURBANCES

A. **Edema** is the presence of excess fluid in the intercellular space. It can be localized or generalized and is caused by:

1. Increased hydrostatic pressure due to venous thrombosis (local) or congestive heart failure (generalized)

2. Hypoalbuminemia, resulting in a decreased colloid osmotic pressure
3. Lymphatic obstruction
4. Renal retention of salt and water

B. **Congestion** is an excessive amount of blood in an area secondary to diminished venous outflow. With increasing stasis, the area acquires a purplish hue.

CLINICAL CORRELATE

Congestion may be seen in purple-discolored legs with chronic venous stasis due to circulatory failure.

C. **Thrombosis** is the solidification of a formed mass of blood components. It requires the interaction of all cells within the vasculature and endothelial cells, as well as circulating elements, such as platelets and the clotting cascade. Clotting is a balance between two opposing forces: those favoring the formation of a stable thrombus and those factors causing breakdown of the clot.

1. **Pathophysiology of thrombosis formation**. Injury to the vascular endothelium causes factors that paradoxically facilitate and inhibit thrombosis.
   a. **Facilitation**
      (1) Exposure of tissue factor from injured cells activates factor VII.
      (2) Exposure of thrombogenic subendothelial collagen activates factor XII.
      (3) Platelets deposit and aggregate due to collagen exposure and generation of thrombi.
   b. **Inhibition**
      (1) Increased prostacyclin (PGI_2) and nitryl (NO_2) inhibit platelet aggregation.
      (2) Synthesis of plasminogen activator promotes fibrinolytic activity.
2. **Sequence of events in thrombogenesis**
   a. Endothelial injury exposes subendothelial collagen.
   b. Platelets adhere, requiring von Willebrand factor and factor VIII; stimulation of the clotting cascade requires thromboplastin release from the endothelium (tissue factor).
   c. Platelets degranulate, releasing ADP and fibrinogen, and synthesize thromboxane A_2.
   d. Platelets aggregate, forming a temporary hemostatic plug. Later, there is formation of a secondary plug enmeshed in fibrin, requiring ADP, thrombin, and thromboxane.
   e. The thrombus retracts and organizes with proliferation of capillaries, fibroblasts, and infiltration by neutrophils and macrophages.
   f. Canalization or formation of a new path for blood flow through the thrombus is accomplished by endothelial

CLINICAL CORRELATE

Classic hemophilia (hemophilia A) is caused by a deficiency or reduced activity of factor VIII; it is characterized by excessive bleeding following trauma and bleeding into the joints (hemarthrosis). PTT is prolonged but bleeding time and PT are normal.

A deficiency in von Willebrand's factor causes von Willebrand's disease, characterized by spontaneous bleeding from mucous membranes and excessive bleeding following trauma. Bleeding time is prolonged but platelet count and PT are normal. PTT is also prolonged because von Willebrand's factor serves as a carrier for factor VIII; von Willebrand's disease therefore results in a functional factor VIII deficiency.

growth over the surface and through the thrombus, resulting in incorporation of the thrombus into the vessel.

3. **Additional factors favoring thrombogenesis**
   a. Endothelial injury releases abundant tissue factor.
   b. Changes in blood flow cause turbulence and stasis. Predisposing factors are sites of turbulence (i.e., vessel bifurcations, valves, past stenoses), atherosclerotic plaques, trauma, certain malignancies, and inflammation.
   c. State of hypercoagulability where there is increased thrombogenesis due to an alteration of the clotting mechanisms (e.g., nephrotic syndrome where more inhibitors than activators are lost).

4. **Morphology of the thrombus.** The head of the thrombus is composed of platelets and fibrin. The tail of the thrombus grows downstream. It consists of red blood cells and fibrin. Lines of Zahn are alternating layers of fibrin, platelets, and RBCs within the tail of the thrombus.
   a. **Mural thrombi** are adherent to the vessel wall. They are not occlusive and affect large vessels, such as the heart and aorta.
   b. **Occlusive thrombi** restrict blood flow most frequently in coronary, cerebral, femoral, iliac, popliteal, and mesenteric vessels. They often overlie an atherosclerotic plaque. Arterial thrombi are often occlusive and result in infarct (e.g., myocardial infarct, strokes), while venous thrombi rarely occlude vessels and tend to embolize.
   c. **Postmortem clot** can be differentiated from a thrombus by the absence of lines of Zahn and by its appearance as a rubbery, coagulated mass that is not attached to the vessel wall but forms a cast of the wall.

5. **Disseminated intravascular coagulation (DIC)** begins with extensive formation of thrombi in the microcirculation, causing consumption of components necessary for hemostasis (i.e., platelets, fibrin, coagulation factors) and activation of the fibrinolytic pathways, leading to a bleeding diathesis. DIC is associated with a diverse array of clinical circumstances, such as amniotic fluid emboli, preeclampsia, Gram-negative sepsis, cancer, trauma, surgery, and burns.

D. **Embolism** is the occlusion of a vessel (either artery or vein) by a mass. Often they are thrombi that have dislodged from their site of formation and have lodged in a distal site occluding blood flow.

1. **Pulmonary emboli** often originate from deep vein thrombosis in the lower legs and less often from deep pelvic veins.

CLINICAL CORRELATE

DIC can be diagnosed by the presence of fibrin split products in the blood, low platelets, and prolonged PT and PTT.

NOTE

An embolus is most likely a thrombus that has dislodged from its site of formation and has traveled to another site.

2. **Systemic emboli** are formed in the arterial circulation; most arise in the heart.
3. **Paradoxical emboli** cross over from the right side to the left side of the heart through septal defects and gain access to the systemic circulation.
4. **Other types of emboli** include gas emboli (e.g., Caisson's disease), fat emboli (e.g., associated with bone fractures), amniotic fluid emboli, bone chips, and tumor cells.

E. **Infarction.** If an artery or vein becomes occluded, then the acute loss of blood supply to the area can result in ischemic necrosis of the tissue. Most infarcts (99%) result from thrombotic or embolic occlusion of an artery or vein. Clinically, common sites of infarction are myocardial, pulmonary, brain, and intestinal tissue. Factors that affect the development of an infarct include:

1. Vascular supply, including collateral circulation
2. Rate of occlusion
3. Vulnerability of the tissue to hypoxia
4. Oxygen-carrying capacity of the blood

F. **Shock** is characterized by vascular collapse. There is a greatly decreased perfusion of both cells and tissue due to reduced blood volume, cardiac output, or vascular tone. Cellular injury is initially reversible, but if anoxia persists, cellular injury becomes progressive, leading to the death of cells and the patient.

1. **Cardiogenic shock** results from myocardial infarction (pump failure).
2. **Hypovolemic shock** results from reduced blood volume from any cause (hemorrhage, fluid loss).
3. **Septic shock** results from bacterial infection, such as Gram-negative septicemia, which causes the release of vasodilatory mediators into the vasculature.
4. **Neurogenic shock** results from anesthesia or spinal cord injury.

NEOPLASMS

A neoplasm is a mass of abnormal tissue whose growth exceeds and is uncoordinated with that of the normal tissues and continues in the same excessive state after cessation of the stimuli that evoked the change.

A. **Definitions**

1. **Anaplasia** is loss of cell differentiation and tissue organization.
2. **Metaplasia** is replacement of one type of adult cell or tissue by another not normally present in that site.

3. **Desmoplasia** is excessive fibrous tissue formation in tumor stroma.
4. **Dysplasia** is abnormal atypical cellular proliferation.
5. **Carcinoma** is malignant tumor of epithelium.
6. **Carcinoma in situ** is malignant tumor of epithelium, which shows no invasion of underlying tissue.
7. **Sarcoma** is nonepithelial (mesenchymal) malignant tumor.
8. **Metastasis** is secondary, discontinuous malignant growth, such as a lung metastasis of a colon carcinoma.
9. **Grade** is an estimate of the cytologic malignancy of a tumor, including the degree of anaplasia and number of mitoses. Nuclear size, chromatin content, nucleoli, and nuclear-to-cytoplasmic ratio are all used.
10. **Stage** is the clinical estimate of the extent of spread of a malignant tumor. Low stage means a localized tumor. Stage rises as tumors spread locally then metastasize.

B. **Tumor markers**

1. **Alpha-fetoprotein (AFP)** is expressed in hepatoma, embryonal cell tumor of the testis, and malignant teratoma.
2. **Carcinoembryonic antigen (CEA)** can be seen in any tumor derived from gut epithelium or in intra-abdominal inflammation (e.g., ulcerative colitis). It is most often elevated in colon and pancreatic cancers. It is also elevated in smokers in the absence of tumor and may be elevated in some carcinomas of the lung.
3. **Beta human chorionic gonadotropin (hCG)** is elevated in choriocarcinoma, hydatidiform mole, and germinoma. It is also elevated in pregnancy, forming the basis of the common pregnancy test.
4. **Prostatic acid phosphatase** elevations are seen in prostate tumors extending outside the capsule of the prostate (stage C or D).
5. **Prostate-specific antigen (PSA)** is also elevated in prostate cancer and in some cases of benign prostatic hyperplasia.
6. **CA-125** is elevated in ovarian cancer.

C. **Ectopic hormone production** causes a paraneoplastic syndrome.

1. **Carcinoid tumors** may produce 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin.
2. **Oat cell tumors of the lung,** derived from neuroendocrine cells, may produce ectopic hormones, most frequently antidiuretic hormone (ADH) or adrenocorticotropic hormone

IN A NUTSHELL

- *Carcinoid tumor → 5-HIAA*
- *Oat cell tumor → ADH, ACTH*
- *Squamous cell tumor → PTH*

(ACTH). Many other small peptide hormones associated with oat cell carcinoma have been described.

3. **Squamous cell carcinoma of the lung** sometimes produces hypercalcemia by releasing a PTH-like molecule.

D. **Metastasis**

1. **Multiple routes to metastasis**
   a. Body cavities and surfaces (e.g., peritoneal, pleural, pericardial) may be directly seeded by tumor cells floating in fluid such as ascites or a pleural effusion.
   b. Hematogenous spread is also quite common for most neoplasms, making the lung a common site for metastases of all kinds of cancer.
   c. Lymphatic spread is the most common route of spread for epithelial carcinomas.
   d. Transplantation via mechanical manipulation (e.g., surgical incision, needle tracts) may occur but is relatively rare.

2. **Sequence of lymphohematogenous spread**
   a. Penetration of blood or lymphatic vessels requires detachment from neighboring cells, amoeboid movement, digestion of basement membranes, and crawling between endothelial cells.
   b. Release and embolization of tumor cells requires detachment from vessel walls and other tumor cells.
   c. Adherence of tumor cells, which become anchored in fibrin mesh, requires cell surface receptors that bind to extracellular matrix proteins.
   d. Penetration of vessel walls in the metastatic site (i.e., lung) also requires altered adhesion molecules and amoeboid movement.
   e. Once tumor cells have crawled out of blood vessels in a new organ, they must survive in a new environment of hormones dissimilar to the cell's original environment. Though millions of cells may be shed from a primary tumor, successful metastasis is relatively rare.

E. **Theories of carcinogenesis**

1. **Somatic mutation** refers to structural changes at the gene or chromosomal level that occur spontaneously or in response to carcinogens after germ cell line maturation. Somatic mutation produces neoplasms that are more often monoclonal than polyclonal.

2. **Aberrant differentiation** in cancer cells occurs in the absence of structural changes, indicating abnormalities of gene regulation affecting growth and differentiation. This may occ

regardless of the stimulus inciting malignant change: chemical, viral, radiation, or spontaneous.

3. **Viral infection** and consequent integration of viral DNA into the host genome may lead to malignant transformation (e.g., hepatitis B genome has been found in hepatoma cells and RNA retroviral DNA copies have been found in some lymphomas).
4. **Cell selection.** Carcinogens favor the expression of a pre-existing population of transformed cells that would not be clinically evident otherwise.

F. **Cytology** is the analysis of individual or clumps of cells to determine the degree of anaplasia. Cytology may be used to analyze cells from any source: uterine, cervix, sputum, plural fluid, ascites, fine needle aspiration, joint fluid, and others. Besides staining cells on slides, cells may be analyzed by flow cytometry, a procedure in which fluorescent antibodies are reacted with cells to determine the surface markers they express. This is most often done in the case of lymphomas and leukemias. Karyotype analysis of tumors is also helpful in showing which chromosomal regions of tumors are abnormal. This helps in classification.

G. **Carcinogenic agents**

1. **Chemical carcinogens** may be divided into two broad groups:
   a. **Direct-acting chemical carcinogens** are mutagens that cause cancer directly, usually by modifying DNA (e.g., alkylating agents).
   b. **Procarcinogens** require metabolic conversion to form active carcinogens. Many strong chemical agents are procarcinogens.
      (1) They require an **initiating agent** and a **promoter**. Exposure to the initiating agent results in an irreversible cellular change, which allows the cells to produce a tumor. The promoter is an agent that increases the tumorigenic process in initiated cells. Cells may not be tumorigenic without previous exposure to an initiating agent. An initiator may cause a mutation, while a promoter causes increased growth rates.
      (2) Potential carcinogens are screened by the Ames test, which detects any mutagenic effects on bacterial cells in culture. Mutagenicity *in vitro* correlates well with carcinogenicity *in vivo*.
2. **Radiation**
   a. **Ultraviolet radiation** produces pyrimidine dimers in DNA, leading to transcriptional errors.

b. **Ionizing radiation** by x-rays and gamma rays causes chain breaks in nucleic acids. When critical genes are mutated, cancer may result.

3. **Oncogenic viruses**
   a. **RNA oncogenic viruses** produce a viral-coded reverse transcriptase allowing synthesis of DNA from a viral RNA template. The DNA can then be integrated into the host genome.
   b. **DNA oncogenic viruses** include papovavirus, adenovirus, and herpesvirus. Infection does not necessarily result in the release of infectious virus. For example, EBV is associated with Burkitt's lymphoma in Africa; HBV is associated with hepatocellular carcinoma. In both cases, viral DNA integrates into the host genome.
4. **Loss of immune regulation.** In patients with immune system dysfunction, an increased number of neoplasms develop, suggesting loss of the surveillance mechanism, which normally destroys neoplastic cells via recognition of "nonself" antigens.

TOXIC AND ENVIRONMENTAL CAUSES OF DISEASE

A. **Lead** (plumbism)

1. **Etiology.** Plumbism is most often due to chronic gradual accumulation; children absorb lead more readily than adults. In children, it may result from ingesting lead-based paint chips, chewing on painted furniture or painted lead pencils, and inhaling highway exhaust. Other sources of lead include improperly glazed ceramic dishes, home-fermented liquor, and contaminated drinking water. The use of lead-free paint and unleaded gasoline is reducing the incidence and severity of plumbism.
2. **Pathogenesis.** Lead inhibits enzymes involved in hemoglobin synthesis (including the inhibition of iron incorporation into tetrapyrrole rings) and inhibits adenyl cyclase activity in the brain and pancreas.
3. **Clinical features.** There is an insidious onset.
   a. **Anemia** is characterized by increased hemolysis, coarse basophilic stippling, and elevated free erythrocyte protoporphyrin. The anemia is hypochromic and microcytic.
   b. **Encephalopathy** is due to diffuse edema, demyelination and neuronal degeneration, which causes delirium, seizures, and coma.

IN A NUTSHELL

Key features of lead poisoning include:

- *Basophilic stippling of RBCs*
- *Peripheral neuropathy*
- *Lead lines in bones*
- *Abdominal colic*

c. **Peripheral neuropathy** is due to myelin degeneration, which predominantly affects motor neurons. The radial nerve is most often affected, leading to wrist drop.
d. **Renal lesions** feature proximal tubular dysfunction, causing glycosuria, aminoaciduria, and hyperphosphaturia (Fanconi's syndrome). It is associated with mineral-containing intranuclear inclusions in proximal tubular cells.
e. **Abdominal colic** is chronic, and often severe.
f. **Lead lines** refer to an accumulation of lead sulfide on the gingival mucosa and epiphyseal radiodensities on x-ray.

B. **Carbon monoxide**

1. **Pathogenesis.** Carbon monoxide combines with hemoglobin to form carboxyhemoglobin, which cannot carry oxygen. The affinity of carbon monoxide for hemoglobin is more than 200 times greater than that of oxygen. Once formed, carbon monoxide is displaced from hemoglobin very slowly.
2. **Types**
   a. **Acute toxicity.** Symptoms of hypoxia are apparent when 30% of hemoglobin is carboxyhemoglobin; coma and death ensue when 60% is carboxyhemoglobin. The blood is cherry red, turning the lips cherry red as well. Carbon monoxide poisoning causes CNS hyperemia, edema, and focal hemorrhages with symmetric degeneration of the basal ganglia. There is loss of consciousness, coma, and death within minutes.
   b. **Chronic toxicity.** Slow poisoning causes systemic pathology with milder CNS changes. Fatty change occurs in the heart, liver, and kidney. The patient can usually recover completely.

C. **Acetaminophen.** Because of its widespread availability, acetaminophen has become a commonly ingested substance in accidental childhood poisonings and in suicide attempts.

1. **Pathogenesis.** Hepatotoxicity is mediated by a toxic reactive metabolite, which, after depleting glutathione stores, binds to hepatocyte macromolecules.
2. **Pathology.** Acetaminophen toxicity causes severe centrilobular hepatic necrosis. The severity correlates with the serum drug level.
3. **Clinical features**. Patients experience nausea, vomiting, abdominal pain, and shock. Hepatic failure is not evident until 2-6 days after ingestion.

NOTE

Cigarette smoke contains carbon monoxide; the percentage of carboxyhemoglobin in smokers is proportionate to the number of cigarettes smoked per day.

D. **Salicylates** are another commonly ingested toxin. They may be ingested accidentally or in suicide attempts.

1. **Pathogenesis.** Initially, direct respiratory stimulation produces a respiratory alkalosis. In addition, the metabolic effects of salicylates cause a metabolic acidosis. Vomiting complicates fluid and electrolyte disturbances. Fatalities are most often due to dehydration and hypokalemia.
2. **Pathology.** Hemorrhagic gastritis, petechiae, systemic hemorrhages, and necrosis of lymphoid germinal centers occur.

E. **Mercury poisoning** is rare.

1. **Pathogenesis.** Mercury inactivates enzymes (particularly cytochrome oxidases) and damages cell membranes.
2. **Types**
   a. **Acute toxicity** causes necrosis of gastric and colonic epithelium, acute renal tubular necrosis, and, possibly, cerebral edema.
   b. **Chronic toxicity** causes excessive salivation, gingivitis, gastritis, renal tubular basement membrane thickening (which causes proteinuria and eosinophilic inclusions), and cerebral (particularly occipital) and cerebellar atrophy.

GENETIC DISORDERS

A. **Autosomal dominant disorders**

1. **Phacomatoses.** Tuberous sclerosis and von Hippel-Lindau disease are transmitted by autosomal dominant inheritance. Neurofibromatosis is transmitted in an autosomal dominant fashion in 50% of cases, and 50% of cases are sporadic mutations.
2. **Familial hypercholesterolemia**
   a. **Clinical features.** Homozygotes have more severe symptoms than heterozygotes, including xanthomas and extensive early atherosclerosis often resulting in myocardial infarction in the second or third decade of life.
   b. **Pathogenesis.** There is a loss of feedback inhibition of cholesterol synthesis caused by decreased or defective low-density lipoprotein (LDL) receptors.
3. **Marfan's syndrome** is a connective tissue abnormality; approximately 85% of cases are autosomal dominant. The incidence of sporadic cases increases with increasing paternal age.
   a. **Pathogenesis** is unclear. There is probably a defect in collagen structure and possibly defective elastin or mucopoly-

charide ground substance due to mutations in the fibrillin glycoprotein gene.

b. **Clinical features** are very variable. They include arachnodactyly (long, spider-like fingers), tall stature, ligamentous laxity, subluxed lens, dissecting aortic aneurysm, usually of the ascending aorta (secondary to cystic medial necrosis of the vessel wall), mitral valve prolapse, and a short life-span, often due to a ruptured aorta.

4. **Familial polyposis coli** (FPC)
   a. Thousands of adenomatous polyps appear, starting in the colorectum and spreading throughout the colon. Polyps first appear in the patient's twenties, become symptomatic in the thirties, and transform to adenocarcinoma by approximately age 40.
   b. Gardner's syndrome has colonic polyps with soft tissue and bone tumors.

5. **Adult polycystic kidney disease** (APKC)
   a. Renal cysts, increasing with age, cause progressively enlarged kidneys. The rate of enlargement of kidneys proceeds at the same rate in affected families.
   b. Hypertension, renal failure, and anemia are the presenting signs, typically starting when patients are in their forties. The age of onset of symptoms also proceeds at the same rate in a given family.
   c. Cysts are also found in the liver, pancreas, spleen, and gonads. There is an increased risk of berry aneurysms and abnormalities of the cardiac valves.

6. **Huntington's disease**
   a. This is a progressive neurologic disorder; the age of onset tends to be the same in affected families.
   b. The onset of symptoms is usually between the ages of 30 and 50 years with involuntary choreic movements (Huntington's chorea), cognitive impairment, and changes in behavior. Death follows after 15-20 years.
   c. It is associated with degeneration of the caudate nucleus.

7. **Wilm's tumor**
   a. This is an embryonal tumor, one of the most common solid tumors in children under 4, involving one or both kidneys and characterized by primitive mesenchyme and immature tubules. Sporadic forms also occur.
   b. Wilm's tumor, aniridia, gonadoblastoma, and mental retardation (WAGR syndrome) are associated with a gene at chromosome 11p13.
   c. This tumor often reaches enormous sizes and can be easily palpated on physical exam as a large abdominal mass.

CLINICAL CORRELATE

Nearly 100% of patients with FPC will get carcinoma of the colon by the fifth decade of life. The treatment of choice is to surgically remove the entire colon, usually in the second or third decade of life.

8. **Retinoblastoma**
   a. This disorder is an embryonal tumor affecting one or both eyes.
   b. Osteosarcoma is associated with familial forms.

B. **Glycogen storage diseases** are inherited via an autosomal recessive pattern.

1. **Type I (von Gierke's disease)** is caused by an enzyme defect in glucose-6-phosphatase.
2. **Type II (Pompe's disease)** is an enzyme defect in lysosomal α–1,4-glucosidase, which affects all organs, especially the heart and brain.
3. **Type III** is due to an enzyme defect in glycogen debranching enzymes, which affects all organs.
4. **Type IV** is due to an enzyme defect in branching enzymes, which affects all organs.
5. **Type V (McArdle's disease)** is due to a defect in striated muscle phosphorylase, which specifically affects striated muscle.
6. **Type VI** is due to a defect in liver phosphorylase, which affects only the liver.

C. **Lysosomal storage diseases**

1. **Mucopolysaccharidoses.** Various lysosomal enzymatic defects lead to the accumulation of glycosaminoglycans throughout the body and brain. All except Hunter's syndrome show autosomal recessive inheritance.
   a. **Pathology.** Storage of glycosaminoglycans occurs mainly in the endothelium, reticuloendothelium, and fibroblasts of the liver, spleen, lymph nodes, vessels, and bone marrow. Balloon cells are formed. Patients also have cardiac valve lesions, hepatosplenomegaly, arterial lesions in coronary and cerebral vessels, and skeletal deformities.
   b. **Types** of mucopolysaccharidoses (MPS) include
      (1) **MPS I H (Hurler's syndrome)**
      (2) **MPS I S (Scheie's syndrome)**
      (3) **MPS I H/S (Hurler-Scheie syndrome)**
      (4) **MPS II (Hunter's syndrome)**
      (5) **MPS III (Sanfilippo's syndrome)**
      (6) **MPS IV (Moruio's syndrome)**

2. **Sphingolipidoses**
   a. **Tay-Sachs disease** (GM_2 gangliosidosis type 1) is due to deficiency of hexosaminidase A, which leads to an accumulation of GM_2 ganglioside, affecting all organs but predominantly the brain, retina, and peripheral nervous system.

(1) **Clinical features.** The onset of symptoms begins at 6 months of age with an exaggerated startle response and progressive mental, motor, and visual deterioration, leading to death by age 3. It can be detected prenatally by amniocentesis. The highest incidence is in Ashkenazic Jews (carrier rate is 1/30).

b. **Gaucher's disease** is due to defects in β-glucocerebrosidase, leading to the accumulation of glucocerebroside, which affects reticuloendothelial cells and the central nervous system.

c. **Niemann-Pick disease** is due to a defect in sphingomyelinase, leading to an accumulation of sphingomyelin and cholesterol in a variety of organs.

(1) **Clinical features.** Eighty percent of cases are type A, which is characterized by extensive CNS and systemic accumulations. Patients suffer from hepatosplenomegaly, xanthomas, fever, vomiting, failure to thrive, neurologic dysfunction, and death by age 2.

(2) **Pathology.** Characteristic findings include enlarged "foamy" cells filled with distended lysosomes containing sphingomyelin.

D. **Other metabolic disorders**

1. **Phenylketonuria** is a disorder resulting from an absence of phenylalanine hydroxylase in homozygotes, which halts the conversion of phenylalanine to tyrosine, resulting in elevated levels of phenylalanine in the blood.

a. **Clinical features.** Infants are normal at birth, but within months, develop an abnormal pattern on EEG with seizures and mental retardation. There is minimal melanin production, causing light hair and skin, and blue eyes. The urine has a musty odor as a result of the urinary excretion of phenylacetic acid. Pathology can be prevented with a special diet free of phenylalanine and supplemented with tyrosine during childhood.

b. **Diagnosis** is by the Guthrie bacterial inhibition assay (routine newborn screening) or by measurement of phenylalanine levels in the blood.

2. **Galactosemia** can result from two different enzyme deficiencies.

a. **Galactokinase deficiency** is a benign disease. The main complication is cataract formation.

b. **Galactose-1-phosphate uridyltransferase deficiency** is a severe form of galactosemia.

NOTE

Aspartame, an artificial sweetener, contains phenylalanine and should be avoided by phenylketonurics.

3. **Albinism** is caused by an enzymatic deficiency that prevents melanin synthesis from tyrosine.
   a. **Types**
      (1) Tyrosinase-negative type is due to a lack of tyrosinase in melanocytes.
      (2) Tyrosinase-positive type, in which tyrosinase is present, is due to a defect in tyrosine uptake.
   b. **Clinical features.** The lack of melanin may be limited to the eye (ocular albinism) or may involve total body pigmentation (oculocutaneous albinism). In the latter, the skin is particularly sensitive to the sun, resulting in premature wrinkling and a tendency to develop solar keratosis, as well as basal cell, squamous cell, and melanocyte carcinomas. Eyes are very photosensitive; visual acuity is decreased.
4. **Cystic fibrosis** is due to an abnormality in chloride channels.
   a. **Diagnosis** may be made by demonstrating elevated chloride and sodium in sweat.
   b. **Clinical features.** Hyperviscous secretions lead to meconium ileus (small bowel obstruction) in 5%-10% of newborns. Patients suffer steatorrhea (from pancreatic insufficiency), pulmonary obstruction, and pneumonia, leading to infection. Secondary cardiac complications follow. Men may be sterile as a result of obstruction of the vas deferens. Cirrhosis of the liver is common.
5. **Alpha$_1$-antitrypsin deficiency**
   a. **Clinical features.** The patient experiences progressive emphysema of the lower lobes of the lungs. This is in contrast to smoking-related emphysema, in which the upper lobes are affected first. Cirrhosis of the liver is seen in some patients.

E. **Disorders of chromosome number or structure**

1. **Trisomic disorders** are usually secondary to a meiotic defect.
   a. **Down's syndrome (trisomy 21)**
      (1) **Incidence.** This defect increases with maternal age. It affects 1 in 2000 live births if maternal age is less than 30 and 1 in 50 live births if maternal age is greater than 45. The incidence of having a second affected child is 1 in 60.
      (2) **Clinical features** include severe mental retardation, characteristic facies (flat nasal bridge, epicanthal folds, oblique palpebral fissures), dysplastic ears, hypotonia, horizontal palmar crease, redundant neck skin, and short trunk. There is also an increased incidence of ventricular septal defect (VSD), acute lymphoblas

leukemia (ALL), and neurologic changes similar to those of Alzheimer's disease.

b. **Edward's syndrome (trisomy 18)**
   (1) **Incidence** is 1 in 5000 births.
   (2) **Clinical features** include severe mental retardation, VSD, micrognathia (a small lower jaw), rocker-bottom feet, low-set ears, prominent occiput, and hypertonia. The average lifespan is 2-3 months.

c. **Patau's syndrome (trisomy 13)**
   (1) **Incidence** is 1 in 6000 births.
   (2) **Clinical features** include microcephaly, severe mental retardation, arrhinencephalia, microphthalmia, cleft lip and palate, VSD, dextrocardia, and polydactly. Death is usually in the neonatal period.

2. **Chromosomal deletions**
   a. **Cri du chat syndrome (5p–)**
      (1) **Pathogenesis.** There is a deletion of the short arm of chromosome 5.
      (2) **Clinical features.** The patient exhibits a cat-like cry up to 1 year of age, severe mental retardation, microcephaly, and epicanthal folds; one in four patients have a VSD. Patients may live to adulthood.
   b. **DiGeorge's syndrome** is caused by absence of the thymus and parathyroids, cardiovascular abnormalities, and low-set ears. It results from a deletion of chromosome 22q11 during development.

3. **Disorders of sex chromosomes**
   a. **Klinefelter's syndrome**
      (1) **Karyotypes.** The most common karyotype is 47,XXY, but other patterns may also be seen.
      (2) **Etiology.** Nondisjunction during meiosis in either the maternal or paternal gamete may result in an extra X chromosome.
      (3) **Incidence** increases with maternal age or irradiation and affects 1 in 800 male births.
      (4) **Clinical features** include testicular atrophy, sterility, a small penis, failure of development of male secondary sexual characteristics, gynecomastia, and mild mental retardation. Mental deficiency is more marked with a greater number of X chromosomes.
      (5) **Laboratory values** show positive X chromatin, azospermia, low serum testosterone, and elevated urinary excretion of FSH.
   b. **Turner's syndrome**
      (1) **Karyotype** is typically 45,XO.

(2) **Incidence** is 1 in 3000 female births.

(3) **Clinical features** may be subtle in mosaics. There is edema during infancy, a web neck, short stature, broad chest with wide-spaced nipples, low hairline, primary amenorrhea, infertility, coarctation of the aorta, streak ovaries, and, usually, abnormal intelligence.

Gastrointestinal Pathology

There are many different organs included in the gastrointestinal system. Gastrointestinal pathology, therefore, includes a wide variety of disorders, from peptic ulcer disease to colorectal cancer to gallstones. Since many of these disorders initially present with similar symptoms (abdominal pain, diarrhea, constipation), it is important to be able to recognize the specific risk factors and signs associated with each disorder. This chapter will discuss the pathology of each organ in the gastrointestinal system, along with the associated risk factors and clinical presentations.

ORAL CAVITY

A. **Congenital malformations** include **cleft lip** and **cleft palate.** Both are generally treated surgically within the first six months of life.

B. **Teeth**

1. **Enamel hypoplasia** is due to a defect in enamel formation, resulting from dysfunction of ameloblasts, which form horizontal bands of discolored, pitted indentations. It may be caused by deficiencies of calcium, phosphorus, vitamins A, C, and D; excess fluoride; infections (e.g., syphilis); hypoparathyroidism, and hypothyroidism.
2. **Pigmentation of developing teeth** may be caused by excess bile pigments in biliary disease, bilirubin in hemolytic anemias, or tetracycline.
3. **Congenital syphilis** leads to malformation of teeth as a result of inflammatory changes in ameloblasts and odontoblasts.

NOTE

The pathologic effect of tetracycline on developing bones and teeth has long been a favorite NDB side effect. Remember: No tetracycline for pregnant women and children under 9 years of age.

C. **Oral mucosa**

1. **Common periodontal diseases**
   a. **Gingivitis** is a chronic inflammation of the gingivae.
   b. **Periodontitis,** or pyorrhea, is gingivitis that has spread into tooth cementum and alveolar bone. Complications include suppurative infection, abscess, and bone resorption.
   c. **Aphthous ulcers** are painful ulcers commonly known as "canker sores." They are not invasive and may be present as a single lesion or in crops, each of which is usually less than 0.5 cm. They often appear during febrile illness or other physical or emotionally stressful situations, and are often found in patients with ulcerative colitis.

2. **Oral manifestations of systemic disease**
   a. **Vitamin deficiencies**
      (1) Vitamin B deficiency leads to **atrophic glossitis** as a result of reduced cell division in the squamous mucosa.
      (2) Vitamin C deficiency causes **bleeding gums** as a result of weakened connective tissue.
   b. **Pregnancy** may cause gingivitis and increased vascularity of the gingivae.
   c. **Hematologic abnormalities**
      (1) Thrombocytopenia may cause petechiae and excess bleeding.
      (2) Leukemia may cause red, boggy gingivae infiltrated by leukemic cells.
      (3) Pernicious anemia causes a smooth, beefy, red tongue due to squamous atrophy.
   d. **Diabetes** may produce dryness of the mucosa and a tendency to form abscesses as a result of impaired microcirculation.
   e. **Addison's disease** leads to generalized excessive pigmentation; Peutz-Jeghers syndrome leads to patchy pigmentation.
   f. **Systemic infectious diseases**
      (1) Scarlet fever, toxic shock syndrome, and Kawasaki's disease cause a **strawberry tongue.**
      (2) Measles produces **Koplik spots,** which are tiny white specks on a red base, found on the buccal mucosa in the prodromal stage of illness.

3. **Infections**
   a. **Necrotizing gingivitis** ("trench mouth") ("ANUG") produces crater-like depressions at the gingival margin. It is painful and causes a fetid odor.
   b. **Herpetic gingivostomatitis** is due to herpes simplex and usually seen in children.

c. **Oral thrush** is caused by ***Candida albicans,*** which produces **white adherent patches.** Thrush is associated with impaired immunity or debilitation, and is commonly seen in patients with AIDS or in patients undergoing chemotherapy.
d. **Herpangina** is due to **coxsackievirus A** and causes vesicular lesions, typically in the pharynx.
e. **Syphilis** may produce a variety of lesions:
(1) Primary syphilis produces chancres on the lips.
(2) Secondary syphilis produces maculopapular eruptions.
(3) Tertiary syphilis produces gummae of the palate and atrophic glossitis.

4. **Keratotic abnormalities**
a. **Lichen planus** appears as white reticulate lesions on the buccal mucosa and tongue.
b. **Leukoplakia** appears as white plaques on oral mucosa, produced by hyperkeratosis of the epithelium. Ten percent of cases of leukoplakia have epithelial dysplasia, a precancerous lesion. Smoking, smokeless tobacco, alcohol abuse, chronic friction, and irritants are predisposing factors.
c. **Erythroplasia** (dysplastic leukoplakia) appears flat, smooth, and red. Significant numbers of atypical epithelial cells are seen microscopically. There is a high risk of malignant transformation.
d. **Hairy leukoplakia** is so named because of its wrinkled surface. Patches occur on the side rather than the middle of the tongue. There are far fewer atypical cells than are seen in erythroplasia. Malignant transformation does not occur, despite its association with HIV and associated infections, including papilloma and Epstein-Barr viruses.

5. **Tumors**
a. **Benign tumors** include hemangiomas, hamartomas, fibromas, lipomas, adenomas, papillomas, neurofibromas, and nevi.
b. **Malignant tumors.** By far, the most common malignant tumor is **squamous cell (epidermoid)** carcinoma. The peak incidence ranges from age 40-70. Squamous carcinoma is associated with **tobacco and alcohol use,** particularly when used together. Pathologically, it may be papillary or ulcerative. The lower lip is the most common site, but cancer of the floor of the mouth, tongue, and buccal mucosa are frequently seen.

D. Salivary glands

1. **Inflammation**
a. **Sialolithiasis** produces a secondary inflammatory reaction to obstruction and the resultant enlargement of ducts by

stones. It may be complicated by actual infection with mouth flora.

b. **Sialadenitis** is a primary inflammatory reaction, but it is not always infectious. It may be part of an autoimmune disease (e.g., Sjögren's syndrome), or the result of bacterial or viral (e.g., mumps) infection.

2. **Tumors.** The parotid gland accounts for more than three-quarters of these tumors, most of which are benign. Of the remainder, more occur in the submandibular gland than in the sublingual, and most of these are malignant. Many are surgically cured, but local recurrence is common.

a. **Pleomorphic adenoma** is generally benign and accounts for approximately three-quarters of all salivary gland tumors. It is composed of multiple epithelial and mesenchymal cell types. Complications may arise due to involvement of cranial nerve VII.

b. **Warthin's tumor** (adenolymphoma) is also benign, occuring almost exclusively in the parotid gland. It is grossly cystic. Microscopic examination reveals cell types suggestive of branchial cleft origin embedded in a lymphoid matrix.

c. **Mucoepidermoid tumors** also occur primarily in the parotid and have a high rate of malignant transformation. The malignant component is usually squamous cell.

d. **Cylindroma** (adenoid cystic carcinoma) is more common in the minor salivary glands found in the oral mucosa, and metastases are more common than in other tumors of the salivary glands. Facial nerve complications are frequent.

(1) **Grossly,** the tumor forms multiple lobules surrounded by a capsule.

(2) **Microscopically,** small cells form glands containing mucoid material.

ESOPHAGUS

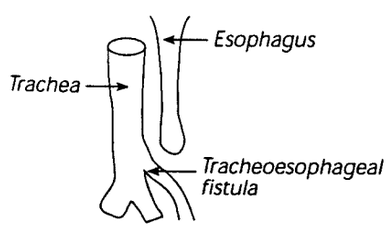
A. **Congenital malformations**

1. A **tracheoesophageal fistula** (the most prevalent esophageal anomaly) occurs most commonly as an upper esophageal blind pouch with a fistula between the lower segment of the esophagus and the trachea. It is associated with hydramnios, congenital heart disease, and other gastrointestinal malformations.

2. Esophageal atresia is associated with VATER syndrome (vertebral defects, anal atresia, tracheoesophageal fistula, and renal dysplasia). It does not usually occur as an isolated anomaly.

CLINICAL CORRELATE

The most common type of tracheoesophageal fistula:



3. **Stenosis** refers to a narrowed esophagus with a small lumen. It may be congenital or acquired, e.g., through trauma or inflammation.

B. Inflammatory disorders

1. **Esophagitis** most often involves the lower half of the esophagus.
   a. **Clinical features.** Patients experience substernal burning associated with regurgitation, mild anemia, dysphagia, hematemesis, and melena. Esophagitis may predispose to esophageal cancer.
   b. **Etiology**
      (1) **Reflux esophagitis** is due to an incompetent lower esophageal sphincter that permits reflux of gastric juice into the lower esophagus.
      (2) **Irritants** such as citric acid, hot liquids, alcohol, smoking, corrosive chemicals, and certain drugs, such as tetracycline, may provoke inflammation.
      (3) **Infectious etiologies** include herpes, CMV, and *C. albicans*. The immunocompromised host is particularly susceptible to infectious esophagitis.
   c. **Pathology**
      (1) **Grossly,** there is hyperemia, edema, inflammation, and superficial necrosis.
   d. **Complications** include ulceration, bleeding, stenosis, and squamous carcinoma.
2. In **Barrett's esophagus,** gastric or intestinal columnar epithelium replaces normal squamous epithelium in response to chronic reflux.

C. Motor disorders. Normal motor function requires effective peristalsis and relaxation of the lower esophageal sphincter.

1. **Achalasia** is a **lack of relaxation of the lower esophageal sphincter (LES),** which may be associated with aperistalsis of the esophagus and increased basal tone of the LES.
   a. **Clinical features.** Achalasia occurs most commonly between the ages of 30 and 50. Typical symptoms are **dysphagia,** regurgitation, aspiration, and chest pain. The lack of motility promotes stagnation and predisposes to carcinoma.
2. **Hiatal hernia** is the herniation of the abdominal esophagus, the stomach, or both, through the esophageal hiatus in the diaphragm.
3. **Scleroderma** is a collagen vascular disease, seen primarily in women, that causes subcutaneous fibrosis and widespread degenerative changes. (A mild variant is known as CREST syn-

NOTE

Reflux occurs when LES pressure decreases enough to allow seepage of stomach contents back into the esophagus.

drome, which stands for **c**alcinosis, **R**aynaud's phenomenon, **e**sophageal dysfunction, **s**clerodactyly, and **t**elangiectasia.) The esophagus is the most frequently involved region of the gastrointestinal tract.

a. **Clinical features** are mainly dysphagia and heartburn due to reflux esophagitis caused by aperistalsis and incompetent LES.

D. **Rings and webs**

1. **Webs** are mucosal folds in the upper esophagus above the aortic arch.
2. **Schatzki rings** are mucosal rings at the squamocolumnar junction below the aortic arch.
3. **Plummer-Vinson syndrome** consists of a triad of **dysphagia, atrophic glossitis,** and **anemia.** Webs are found in the upper esophagus. The syndrome is associated specifically with iron deficiency anemia and sometimes hypochlorhydria. Patients are at increased risk for carcinoma of the pharynx or esophagus.

E. **Mallory-Weiss tears** refers to small mucosal tears at the gastroesophageal junction secondary to **recurrent forceful vomiting,** usually seen in **alcoholics.** The tears occur along the long axis and result in **hematemesis** (sometimes massive).

F. **Esophageal varices** are dilated tortuous vessels of the esophageal venous plexus resulting from **portal hypertension.** When portal blood pressure increases, collateral circulation through the coronary veins to the esophageal veins and then to the azygous system develops, yielding vessel engorgement. Portal hypertension is most often caused by hepatic cirrhosis. Another common cause is obstructive thrombosis of the portal or splenic vein. Esophageal varices are prone to bleeding and ulceration, which may be life-threatening, especially in cirrhotics.

G. **Diverticula** are sac-like protrusions of one or more layers of the pharyngeal or esophageal wall.

H. **Tumors**

1. **Benign** tumors are rare.
2. **Carcinoma of the esophagus** most commonly occurs after age 50 and has a male:female ratio of 4:1.
   a. **Incidence.** In the United States, the incidence is much higher in African Americans than in Caucasians.
   b. **Etiology.** It is associated with smoking, alcohol ingestion, nitrosamines in food, achalasia, webs, rings, diverticula, Barrett's esophagus, and deficiencies of vitamins A and C, riboflavin, and some trace minerals.

IN A NUTSHELL

Plummer-Vinson syndrome:

- *Dysphagia*
- *Glossitis*
- *Iron deficiency anemia*
- *Esophageal webs*

IN A NUTSHELL

Esophageal varices are often due to portal hypertension. They may bleed or ulcerate, which can be life-threatening.

IN A NUTSHELL

Mallory-Weiss tears vs. esophageal varices:

While both are associated with alcohol abuse and can present with hematemesis, Mallory-Weiss tears typically occur acutely as a result of retching/vomiting. Esophageal varices result from portal hypertension and will usually present with a more significant bleeding episode.

c. **Clinical features** include dysphagia (first to solids), retrosternal pain, anorexia, weight loss, melena, and symptoms secondary to metastases.

d. **Pathology**

(1) 50% occur in the middle third of the esophagus, 30% in the lower third, and 20% in the upper third. Most esophageal cancers are squamous cell carcinomas. Adenocarcinomas arise mostly out of Barrett's esophagus.

e. **Prognosis** is poor. Fewer than 10% of patients survive 5 years, usually because diagnosis is made at a late stage. The most common sites of metastasis are the liver and lung. The combination of cigarette **smoking** and **alcohol** is particularly causative for esophageal cancer (over 100× risk compared to nondrinkers/nonsmokers).

STOMACH

A. Congenital malformations

1. **Pyloric stenosis**

a. **Clinical features. Projectile vomiting** 3-4 weeks after birth associated with a **palpable "olive" mass** in the epigastric region is observed.

b. **Pathology** shows hypertrophy of the muscularis of the pylorus and failure to relax.

2. **Diaphragmatic hernias** are due to weakness in or absence of parts of the diaphragm, allowing herniation of the abdominal contents into the thorax.

CLINICAL CORRELATE

Pyloric stenosis is congenital hypertrophy of pyloric muscle, presenting with projectile vomiting and requiring surgical treatment.

B. Inflammation

1. **Acute gastritis** (erosive)

a. **Etiology.** Alcohol, aspirin and other NSAIDs, smoking, shock, steroids, and uremia may all cause disruption of the mucosal barrier, leading to inflammation.

b. **Clinical features.** Patients experience heartburn, epigastric pain, nausea, vomiting, hematemesis, and even melena.

2. **Chronic gastritis** (nonerosive) may lead to atrophic mucosa with lymphocytic infiltration.

a. **Types**

(1) **Fundal (Type A) gastritis** is often **autoimmune** in origin. It is the type associated with **pernicious anemia** and, therefore, achlorhydria and intrinsic factor deficiency.

(2) **Antral (Type B) gastritis** is most commonly caused by *Helicobacter pylori* and is the most common form of

CLINICAL CORRELATE

Gastritis may be acute (NSAIDs, alcohol, stress, etc.) or chronic (autoimmune or H. pylori).

chronic gastritis in the U.S. *H. pylori* is also responsible for proximal duodenitis in regions of gastric metaplasia.

b. **Clinical features.** The patient may be asymptomatic or suffer epigastric pain, nausea, vomiting, and bleeding. Gastritis may predispose to peptic ulcer disease, probably related to *H. pylori* infection.

3. **Peptic ulcers** are usually chronic, isolated ulcers observed in areas bathed by pepsin and HCl; they are the result of mucosal breakdown (Figure 2-1).
   a. **Common locations** are the proximal duodenum, the stomach, and the esophagus, often in areas of Barrett's esophagus.
   b. **Etiology.** There are several important etiologic factors. Duodenal ulcers occur predominantly in patients with excess acid secretion, while gastric ulcers usually occur in patients with lower than average acid secretion. Other predisposing conditions include smoking, cirrhosis, pancreatitis, hyperparathyroidism, and *H. pylori* infection. Aspirin, steroids, and NSAIDs are known to be assoicated with peptic ulcer disease. Next to *H. pylori* colonization, aspirin or NSAID ingestion is the most common cause of peptic ulcer.
   c. **Clinical features.** Patients experience **episodic epigastric pain.** Duodenal and most gastric ulcers are relieved by food or antacids. Approximately one-fifth of gastric ulcer patients get no relief from eating or experience pain again within 30 minutes.
   d. **Pathology.** Benign peptic ulcers are well-circumscribed lesions with a loss of the mucosa, underlying scarring, and sharp walls.
   e. **Complications** include hemorrhage, perforation, obstruction, and pain. Duodenal ulcers do not become malignant. Gastric ulcers do so only rarely; those found to be malignant likely originated as a cancer that ulcerated.
   f. **Diagnosis** is made by upper gastrointestinal series, endoscopy, and biopsy to rule out malignancy or to demonstrate the presence of *H. pylori.*
4. **Stress ulcers** are superficial mucosal ulcers of the stomach or duodenum or both. Stress may be induced by burns, sepsis, shock, trauma, or increased intracranial pressure.

CLINICAL CORRELATE

H. pylori has now been shown to be associated with gastritis, peptic ulcer disease, gastric adenocarcinoma, and some gastric lymphomas.

CLINICAL CORRELATE

Recent evidence raises the importance of H. pylori in the pathogenesis of peptic ulcer disease. Modification of acid secretion alone does not effect a lasting remission, but coupling this with antibiotic therapy that eradicates H. pylori is apparently curative in most patients.

IN A NUTSHELL

Gastric ulcers may develop into, or develop from, a malignancy. Duodenal ulcers are never malignant.

Figure 2-1. Peptic ulcer disease of the stomach (gross).

C. **Tumors**

1. **Benign**
   a. **Leiomyoma,** often multiple, is the most common benign neoplasm of the stomach. Clinical features include bleeding, pain, and iron deficiency anemia.
   b. **Gastric polyps** are due to proliferation of the mucosal epithelium.
2. **Malignant tumors**
   a. **Carcinoma**
      (1) **Etiology.** Primary factors include genetic predisposition and diet; other factors include hypochlorhydria, pernicious anemia, atrophic gastritis, adenomatous polyps, and exposure to nitrosamines. *H. pylori* are also implicated.
      (2) **Clinical features.** Stomach cancer is usually asymptomatic until late, then presents with anorexia, weight loss, anemia, epigastric pain, and melena. **Virchow's node** is a common site of metastasis.
      (3) **Pathology.** Symptomatic late gastric carcinoma may be expanding or infiltrative. In both cases the prognosis is poor (approximately 10% 5-year survival), and metastases are frequently present at the time of diagnosis. **Adenocarcinomas** are most common.

CLINICAL CORRELATE

Virchow's node is a left supraclavicular lymph node. Its presence suggests metastatic stomach carcinoma.

NOTE

An infiltrating gastric carcinoma with a diffuse fibrous response is called a linitis plastica (leather-bottle stomach).

b. **Gastrointestinal lymphomas** may be primary in the gastrointestinal tract as solitary masses.
c. **Sarcoma** is a rare, large, ulcerating mass that extends into the lumen.
d. **Metastatic carcinoma. Krukenberg's tumor** is an ovarian metastasis from a gastric carcinoma.
e. **Kaposi's sarcoma.** The stomach is the most commonly involved GI organ in Kaposi's sarcoma. It primarily occurs in homosexual men, appearing as hemorrhagic polypoid, or umbilicated nodular lesions, typically in a submucosal location. It rarely causes symptoms.

SMALL INTESTINE

A. **Congenital anomalies**

1. **Meckel's diverticulum** (a true diverticulum) is due to persistence of the **omphalomesenteric vitelline duct.**
2. **Atresia** is a congenital absence of a region of bowel, leaving a blind pouch or solid fibrous cord.
3. **Stenosis** refers to a narrowing of any region of the gastrointestinal tract, which may cause obstruction.
4. **Duodenal diverticula** are areas of congenital weakness permitting saccular enlargement. The duodenum is the most common region of the small bowel to contain diverticula.
5. **Diverticula of jejunum and ileum** are herniations of mucosa and submucosa at points where the mesenteric vessels and nerves enter.

B. **Infections**

1. **Bacterial enterocolitis** may be caused by the **ingestion of preformed bacterial toxins,** producing symptoms ranging from severe but transient nausea, vomiting, and diarrhea (*Staphylococcus aureus* toxin) to lethal paralysis (*Clostridium botulinum* toxin). **Ingestion of toxigenic bacteria** with colonization of the gut (e.g., *Vibrio cholera,* toxigenic *E. coli,* various species of *Campylobacter jejuni,* Shigella, Salmonella, Yersinia, and many others) is another potential cause.
2. **Nonbacterial gastroenterocolitis**
   a. Viral
      (1) Rotavirus (children)
      (2) Parvovirus (adults)
   b. Fungal—Candida

c. Parasitic
   (1) *Entamoeba histolytica*
   (2) *Giardia lamblia*

3. **In HIV patients.** Causes of infectious diarrhea in HIV patients include Cryptosporidium, Microsporidia, *Isospora belli*, CMV, and *M. avium-intracellulare.*

C. **Malabsorption** is defined as impaired intestinal absorption of dietary constituents. Clinical features include diarrhea, steatorrhea, weakness, lassitude, and weight loss. Steatorrhea results in deficiency of fat-soluble vitamins (A, D, E, K) and calcium.

1. **Celiac sprue**
   a. **Etiology.** Celiac sprue (nontropical sprue or gluten enteropathy) is caused by an allergic, immunologic, or toxic reaction to the gliadin component of gluten. There is a genetic predisposition.
2. **Tropical sprue**
   a. **Etiology.** Tropical sprue is of unknown etiology, but may be caused by enterotoxigenic *E. coli.*
3. **Disaccharidase deficiency** is due to a deficiency of brush border enzymes. **Lactase deficiency** is most common.

CLINICAL CORRELATE

Lactase deficiency leads to milk intolerance, with symptoms of bloating, diarrhea, and cramping following ingestion of dairy products.

D. **Vascular abnormalities** often lead to ischemic bowel disease.

1. **Transmural infarction** is more common in the small intestine, which does not have the rich collaterals of the colon.
   a. **Etiology**
      (1) **Thrombosis or embolism of the superior mesenteric artery** accounts for approximately 50% of cases. The thrombosis is most often secondary to atherosclerosis, but emboli may arise from cardiac sources or atherosclerotic plaques higher in the aorta. The inferior mesenteric artery accounts for approximately 25% of cases.
      (2) **Venous thrombosis** accounts for 25% of cases. It typically occurs post CHF, in polycythemia, in hypercoagulable states, or in inflammations of the abdomen.
      (3) **Internal hernias** can strangulate entrapped loops of bowel. They can occur congenitally in children and young adults, or as a result of abdominal surgery (peritoneal adhesions) in adults.
   b. **Clinical features.** There is a 50-75% mortality rate. Infarction of the bowel usually occurs after age 60 and presents as an **acute abdomen** with abdominal pain, nausea, and vomiting.

CLINICAL CORRELATE

Small bowel infarcts are often due to thromboembolic events, and present as an acute abdomen.

E. **Obstructive lesions**

1. **Hernias** cause 15% of small intestinal obstruction. They are due to a protrusion of a serosa-lined sac through a weakness in the wall of the peritoneal cavity. They occur most commonly at the inguinal and femoral canals, at the umbilicus, and with scars. They may lead to entrapment, incarceration, and strangulation of the bowel.

F. **Tumors** of the small bowel account for only 5% of gastrointestinal tumors.

1. **Benign tumors** in descending order of frequency include: leiomyomas, lipomas, adenomas (polyps), angiomas, and fibromas. Adenomatous polyps are most common in the stomach and duodenum and may be single or multiple, sessile or pedunculated. The larger the polyp, the greater the incidence of malignant transformation.
2. **Malignant tumors,** in descending order of frequency, include: endocrine cell tumors, lymphomas, adenocarcinomas, and leiomyosarcomas.

LARGE INTESTINE (COLON)

A. **Congenital anomalies**

1. **Hirschsprung's disease** produces a **markedly distended colon,** usually proximal to the rectum.
2. **Imperforate anus** is due to a failure of perforation of the membrane that separates the endodermal hindgut from the ectodermal anal dimple.

B. **Benign conditions**

1. **Diverticular disease** refers to multiple outpouchings of the colon.
   a. **Incidence.** Diverticular disease is present in 30%-50% of adult autopsies in the United States. There is a higher incidence with increasing age.
   b. **Pathogenesis.** Herniation of mucosa and submucosa through weak areas of the gut wall where arterial vasa recta perforate the muscularis is a characteristic pathologic finding of the disease.
   c. **Clinical features**
      (1) **Diverticulosis** is often asymptomatic, but may present with pain and/or **rectal bleeding.**
      (2) In contrast, **diverticulitis** presents with pain and fever. It is distinguished from diverticulosis by the presence of **inflammation,** which may or may not cause symptoms

NOTE

Since only two layers of the gut wall are involved, the outpouchings are technically ***pseudodiverticula.***

When symptomatic, the patient experiences colicky left lower abdominal pain, change in bowel habits, and melena, so-called "left-sided appendicitis."

d. **Pathology**

(1) **Grossly,** diverticula are seen most frequently in the **sigmoid colon.**

C. Inflammatory diseases

1. **Crohn's disease,** or regional enteritis, causes a segmental, recurrent, **granulomatous inflammatory disease** of the bowel. It most commonly involves the terminal ileum and colon but may involve any part of the gastrointestinal tract. There is a familial disposition.

   a. **Etiology.** There is probably a similar etiology for both Crohn's disease and ulcerative colitis, which together are called **inflammatory bowel disease.** The following possible etiologies have been considered: infectious; immunologic (both antibody-mediated and cell-mediated); deficiencies of suppressor cells; and nutritional, hormonal, vascular, and traumatic factors.

   b. **Clinical features.** Crohn's disease usually begins in early adulthood and is common in Ashkenazic Jews. Patients present with colicky pain, diarrhea, weight loss, malaise, malabsorption, low-grade fever, and melena. There is typically a remitting and relapsing course. If the involved bowel is resected, lesions frequently develop in previously uninvolved regions of the bowel.

   c. **Pathology. Crohn's disease** has a very characteristic pathology.

   (1) **Grossly,** there are **segmental areas (skip lesions)** of involvement, most commonly in the terminal ileum.

2. **Ulcerative colitis** is a chronic relapsing disease characterized by ulcerations, predominantly of the rectum and left colon, but which may affect the entire colon and occasionally the terminal ileum.

   a. **Incidence** is higher in Caucasians than in Blacks, and is also more frequent in women than in men. The typical age of onset ranges from 12-35 years of age. There is a definite familial predisposition.

   b. **Etiology.** Etiologic theories are similar to those for Crohn's disease. Some inflammatory bowel disease has microscopic features of both ulcerative colitis and Crohn's disease.

   c. **Clinical course** is characterized by relapsing **bloody mucus diarrhea,** which may lead to dehydration and electrolyte imbalances, lower abdominal pain, and cramps. There is an

CLINICAL CORRELATE

Crohn's disease presents with pain, diarrhea, weight loss, malabsorption, and malaise.

IN A NUTSHELL

Ulcerative colitis is a chronic disease characterized by colonic mucosal and submucosal ulcerations. Bloody diarrhea is the hallmark clinical presentation.

IN A NUTSHELL

Crohn's disease:

- *Anywhere along GI tract (usually terminal ileum and cecum)*
- *Skip lesions (not continuous)*
- *Transmural*
- *Cobblestone mucosa*

Ulcerative colitis:

- *Continuous involvement from rectum proximally*
- *Limited to mucosa and submucosa (not transmural; no fissures or fistulas)*
- *Pseudopolyps*
- *Greater risk of developing colon adenocarcinoma than in Crohn's*

increased incidence of carcinoma of the colon, up to 50% after 25 years with the disease.

d. **Pathology**

(1) **Grossly,** the disease almost always involves the rectum. It may extend proximally to involve part of the colon or its entirety. There are superficial mucosal ulcers, shortening of the bowel, narrowing of the lumen, **pseudopolyps,** and **backwash ileitis.**

(2) In contrast to Crohn's disease, the inflammation is usually **confined to the mucosa and submucosa.**

3. **Pseudomembranous colitis** is an inflammatory process characterized by a pseudomembranous exudate coating the colonic mucosa.

a. **Pathogenesis.** The syndrome is associated with antibiotic use (especially **clindamycin**), allowing proliferation of ***Clostridium difficile,*** which produces an exotoxin.

b. **Clinical features** include diarrhea that is often bloody, fever, and leukocytosis.

c. **Diagnosis** is made by identification of *C. difficile* and toxin in stool.

d. **Treatment** includes stopping the original antibiotic and starting oral **vancomycin or metronidazole.** This disease is often a terminal complication in immunosuppressed patients.

D. **Vascular lesions**

1. **Hemorrhoids** are variceal dilatations of the anal and perianal venous plexus. They are caused by elevated intra-abdominal venous pressure, often from constipation and pregnancy and are occasionally due to portal hypertension, where they are associated with esophageal varices. Hemorrhoids may undergo thrombosis, inflammation, and recanalization. **External hemorrhoids** are due to dilatation of the inferior hemorrhoidal plexus, while **internal hemorrhoids** are due to dilatation of the superior hemorrhoidal plexus.

E. **Polyps** are mucosal protrusions.

1. **Hyperplastic polyps** comprise 90% of all polyps. They are nonneoplastic and occur mostly in the rectosigmoid colon.

a. **Grossly,** they form smooth, discrete, round elevations.

2. **Adenomatous polyps** are true neoplasms. There is a higher incidence of cancer in larger polyps and in those containing a greater proportion of villous growth.

a. **Tubular adenomas** (pedunculated polyps) make up 75% of adenomatous polyps. They may be sporadic or familial. For

sporadic polyps, the ratio of men to women is 2:1. The average age of onset is 60.

(1) **Grossly,** most occur in the left colon. Cancerous transformation (i.e., invasion of the lamina propria or the stalk) occurs in approximately 4% of patients.

b. **Villous adenomas** are the largest, least common polyps, and are usually sessile. About one-third are cancerous. Most are within view of the colonoscope.

(1) **Grossly,** they form "cauliflower-like" sessile growth 1-10 cm in diameter, which are broad-based and have no stalks.

3. **Familial polyposis** is due to deletion of a gene located on chromosome 5q.

a. **Familial multiple polyposis** (adenomatous polyposis coli) shows **autosomal dominant inheritance** and the appearance of polyps during adolescence; polyps start in the rectosigmoid area and spread to cover the entire colon. The polyps are indistinguishable from sporadic adenomatous polyps. Virtually all patients develop cancers. When diagnosed, total colectomy is recommended.

b. **Gardner's syndrome** refers to colonic polyps associated with other neoplasms (e.g., in skin, subcutaneous tissue, bone) and desmoid tumors. The risk of colon cancer is nearly 100%.

c. **Peutz-Jeghers syndrome** presents with polyps on the entire gastrointestinal tract (especially the small intestine) associated with **melanin pigmentation** of the buccal mucosa, lips, palms, and soles. The polyps are hamartomas and are not premalignant. Peutz-Jeghers syndrome shows autosomal dominant inheritance.

d. **Turcot's syndrome** is characterized by colonic polyps associated with brain tumors (i.e., gliomas, medulloblastomas).

F. **Malignant tumors**

1. **Adenocarcinoma** is the histologic type of 98% of all colonic cancers. Both environmental and genetic factors have been identified.

a. **Incidence** is very high in urban, Western societies. It is the third most common tumor in both women and men. The peak incidence is in the seventh decade of life.

b. **Pathogenesis** is associated with villous adenomas, ulcerative colitis, Crohn's disease, familial polyposis, and Gardner's syndrome. Incidence is possibly related to high-meat intake, low-fiber diet, and deficient vitamin intake. A

number of chromosomal abnormalities have been associated with the development of colon cancer.

c. **Clinical features** include rectal bleeding, change in bowel habits, weakness, malaise, and weight loss in high-stage disease. The tumor spreads by direct extension and metastasis to nodes, liver, lung, and bones. **Carcinoembryonic antigen** (CEA) is a tumor marker that helps to monitor tumor recurrence after surgery or tumor progression in some patients.

d. **Pathology**

(1) **Grossly,** 75% of tumors occur in the rectum and sigmoid colon (Figure 2-2).

(2) **Microscopically,** these tumors are typical mucin-producing adenocarcinomas.

2. **Squamous cell carcinoma** forms in the anal region. It is often associated with papilloma viruses and its incidence is rising in homosexual males with AIDS.

IN A NUTSHELL

Adenocarcinoma is the most common type of colon cancer. It can present with rectal bleeding, changed bowel habits, weight loss, and other systemic symptoms.

Figure 2-2. Carcinoma of the colon (gross).

APPENDIX

A. **Inflammation**

1. **Appendicitis** is almost always acute in onset.

a. **Pathogenesis.** Obstruction leads to mucus retention and distention, compromise of blood supply, and subsequent bacterial infection.

b. **Clinical features** typically include initial periumbilical pain that then localizes to the right lower quadrant, accompanied by anorexia, nausea, vomiting, fever, and moderate leukocytosis.

B. **Mucocele of the appendix** is dilatation of the appendix caused by mucin accumulation.

C. **Tumors.** Carcinoids are most common; carcinomas are rare. Primary lymphoma is occasionally seen.

PERITONEUM

A. **Peritonitis** is inflammation of the peritoneum.

1. **Sterile peritonitis** may be caused by bile, pancreatic enzymes, and foreign materials.
2. **Bacterial peritonitis.** In this condition, the membrane becomes dull with an accumulation of turbid fluid. The exudate becomes frankly purulent and may cause abscesses and adhesions after healing.

B. **Mesenteric cysts** are cysts within the mesenteries or attached to the peritoneum. They are usually single and of variable size. Most are benign.

C. **Sclerosing retroperitonitis** is a dense fibrotic proliferation of the retroperitoneum. The etiology is unknown.

D. **Tumors** of the peritoneum are usually malignant.

1. **Primary mesotheliomas** are rare. Eighty percent are associated with **asbestos exposure.**
2. **Secondary (metastatic)** tumors are common; most are ovarian or pancreatic. Implants from metastatic teratomas sometimes mature and lose their capacity to invade or metastasize further.

CLINICAL CORRELATE

Appendicitis pain begins periumbilically and then localizes to the actual anatomic site of the appendix.

EXOCRINE PANCREAS

BRIDGE TO ENDOCRINE SYSTEM

Disorders of the endocrine pancreas are discussed in the Endocrine Pathology chapter of this book.

A. **Congenital anomalies**

1. **Ectopic pancreatic tissue** most commonly occurs in the stomach, duodenum, jejunum, Meckel's diverticulum, and ileum. It may be either asymptomatic or cause obstruction, bleeding, or intussusception.
2. **Annular pancreas** is a ring of pancreatic tissue that encircles the duodenum and may cause duodenal obstruction.

B. **Cystic fibrosis** is a systemic disorder of exocrine gland secretion, presenting during infancy or childhood.

1. **Incidence** is 1:2500 in Caucasians; it is less common in Blacks, and extremely rare in Asians.
2. **Pathogenesis.** Cystic fibrosis shows autosomal recessive transmission; heterozygotes are unaffected. It results in a **defective chloride channel,** which leads to secretion of **very thick mucus.**
3. **Characteristics**
   a. Tissues other than exocrine glands are normal, and glands are structurally normal until damaged by cystic fibrosis.
   b. The only characteristic biochemical abnormalities are **an elevation of sodium and chloride levels in sweat,** and a decrease in water and bicarbonate secretion from pancreatic cells, resulting in a viscous secretion.
4. **Clinical features**
   a. Fifteen percent of cases present with **meconium ileus.**
   b. Most cases present during the first year with **steatorrhea** (with resultant deficiencies of vitamins A, D, E, and K), abdominal distention, and **failure to thrive.**
   c. Complications are also related to pulmonary infections and obstructive pulmonary disease as a result of viscous bronchial secretions.
5. **Pathology**
   a. There is **mucus plugging of the pancreatic ducts** with cystic dilatation, fibrous proliferation, and atrophy. Similar pathology develops in salivary glands.
   b. **Lungs.** Mucus impaction leads to bronchiolar dilatation and secondary infection.
   c. The **gastrointestinal tract** shows obstruction caused by mucus impaction in the intestines with areas of biliary cirrhosis, resulting from intrahepatic bile duct obstruction.
6. **Diagnosis** depends on demonstrating a **"sweat test"** abnormality associated with at least one clinical feature. In the sweat test, high levels of chloride are demonstrated.

CLINICAL CORRELATE

CF presents with steatorrhea and frequent respiratory infections in young children (often Pseudomonas). An abnormal sweat chloride test will confirm the diagnosis.

7. **Prognosis.** Mean survival is age 20; mortality is most often due to pulmonary infections.

. **Degenerative changes**

1. **Iron pigmentation** (e.g., from hemochromatosis) may be deposited within acinar and islet cells and may cause insulin deficiency.
2. **Atrophy**
   a. **Ischemic atrophy** is due to atherosclerosis of pancreatic arteries and is usually asymptomatic.
   b. **Obstruction of pancreatic** ducts affects only the exocrine pancreas, which becomes small, fibrous, and nodular.

D. **Acute hemorrhagic pancreatitis** presents as a diffuse necrosis of the pancreas caused by the release of activated pancreatic enzymes. Associated findings include fat necrosis and hemorrhage into the pancreas.

1. **Incidence.** This disorder is most often associated with alcoholism and biliary tract disease. It affects middle-aged individuals and often occurs after a large meal or excessive alcohol ingestion; approximately 50% of patients have gallstones.
2. **Pathogenesis.** There are four theories.
   a. **Obstruction of the pancreatic duct** causes an elevated intraductal pressure, which results in leakage of enzymes from small ducts. Obstruction may be caused by a gallstone at the ampulla of Vater; chronic alcohol ingestion may cause duct obstruction by edema.
   b. **Hypercalcemia** may cause activation of trypsinogen; its mechanism is unclear. Pancreatitis occurs in 20% of patients with hyperparathyroidism.
   c. **Direct damage to acinar cells** may occur by trauma, ischemia, viruses, and drugs.
   d. **Hyperlipidemia** may occur as a result of exogenous estrogen intake and alcohol ingestion.
3. **Clinical features** are typically the sudden onset of acute, continuous, and intense **abdominal pain, often radiating to the back** and accompanied by nausea, vomiting, and fever. This syndrome frequently results in **shock.** Laboratory values reveal **elevated amylase** (**lipase** elevated after 3-4 days) and leukocytosis. Hypocalcemia is a poor prognostic sign.

E. **Chronic pancreatitis** refers to remitting and relapsing episodes of mild pancreatitis, causing progressive pancreatic damage.

1. **Incidence** is similar to acute pancreatitis. It is also seen in patients with ductal anomalies. Almost half the cases occur without known risk factors.

IN A NUTSHELL

Chronic pancreatitis presents with steatorrhea, diabetes, and abdominal mass (pseudocyst).

2. **Pathogenesis** is unclear; possibly, there is excess protein secretion by the pancreas, causing ductal obstruction.
3. **Clinical features** include flareups precipitated by alcohol, overeating, and drugs. Attacks are characterized by upper abdominal pain, tenderness, fever, and jaundice. Laboratory values reveal **elevated amylase** and **alkaline phosphatase.** X-rays reveal **calcifications in the pancreas.** Chronic pancreatitis may result in pseudocyst formation, diabetes, and steatorrhea.

F. **Carcinoma of the pancreas**

1. **Incidence**
   a. Carcinoma of the pancreas accounts for approximately 5% of all cancer deaths. Increased risk is associated with smoking, high-fat diet, and chemical exposure. There is a higher incidence in the elderly, Blacks, males, and diabetics.
2. **Clinical features**
   a. The disease is usually asymptomatic until late in its course.
   b. Manifestations include weight loss, abdominal pain frequently radiating to the back, weakness, malaise, anorexia, depression, and ascites.
   c. There is jaundice in half of the patients who have carcinoma of the head of the pancreas.
   d. **Courvoisier's law** holds that painless jaundice with a palpable gallbladder is suggestive of pancreatic cancer.
3. **Pathology.** Carcinomas arise in ductal epithelium. Most are adenocarcinomas.
   a. **Carcinoma of the head of the pancreas** accounts for 60% of all pancreatic cancers.
   b. Carcinoma of the body (20%) and tail (5%) produce large, indurated masses that spread widely to the liver and lymph nodes.
   c. In 15% of patients, carcinoma involves the pancreas diffusely.
4. **Complications** include **Trousseau's syndrome,** a migratory thrombophlebitis that occurs in 10% of patients.
5. **Prognosis** is very poor. If resectable, the 5-year survival rate is less than 5%. The usual course is rapid decline; on average, death occurs 6 months after the onset of symptoms.

IN A NUTSHELL

Chronic pancreatitis presents with steatorrhea, diabetes, and abdominal mass (pseudocyst).

G. **Cysts**

1. **Congenital cysts** frequently occur with cystic disease of the liver and kidney. They are usually multiple.
2. **Pseudocysts** occur as sequelae of pancreatitis or trauma. They are caused by loculation of fluid (suppurative, hemorrhagic, or necrotic debris).

3. **Cystadenomas** and **cystadenocarcinomas** are neoplastic cysts. These are true cysts, lined by epithelium with papillary projections.

LIVER

A. Congenital malformations

1. **Accessory lobes** are most often inferior. They are not associated with any specific disease process.
2. **Congenital cystic disease** is associated with congenital polycystic disease of the kidneys and is asymptomatic.
3. **Congenital hepatic fibrosis** is a disorder demonstrating autosomal recessive inheritance. It is characterized by periportal fibrosis, resulting in hepatosplenomegaly and esophageal varices.
4. **Extrahepatic biliary atresia** causes cholestasis, which results in cirrhosis and portal hypertension.
5. **Intrahepatic biliary atresia** results in a diminished number of bile ducts.

B. **Jaundice,** or **icterus,** is caused by excess **bilirubin accumulation** in the skin and sclerae, producing a yellow discoloration of these tissues. Icterus is visible when the serum bilirubin exceeds 2 mg/dl. In unconjugated hyperbilirubinemia, bilirubin is not excreted into the urine because of tight protein binding in serum. In conjugated hyperbilirubinemia, small amounts of bilirubin are excreted in the urine because it is less tightly protein bound.

C. Hepatic failure

1. **Etiology. Chronic hepatic disease** (e.g., chronic active hepatitis or alcoholic cirrhosis) is the most common cause of hepatic failure although acute liver disease may also be responsible.
   a. **Widespread liver necrosis** may be seen with carbon tetrachloride and acetaminophen toxicity. Widespread steatosis is seen in **Reye's syndrome,** a cause of acute liver failure most often seen in children with a recent history of aspirin ingestion for an unrelated viral illness.
   b. **Massive necrosis** may also be seen in acute viral hepatitis, after certain anesthetic agents, and in shock from any cause.
2. **Clinical features.** Hepatic failure causes jaundice, musty odor of breath and urine, encephalopathy, renal failure (either by simultaneous toxicity to the liver and kidneys or the hepatorenal syndrome), palmar erythema, spider angiomas, gyneco-

mastia, testicular atrophy (secondary to impaired estrogen degradation), prolonged prothrombin time (impaired hepatic synthesis of coagulation factors), weight loss, muscle wasting, pruritus, malabsorption, hypoalbuminemia, hypercholesterolemia, and anemia.

D. **Hemodynamic and vascular abnormalities**

1. **Chronic passive congestion** is associated with right heart failure and is a common postmortem finding.
2. **Central hemorrhagic necrosis** may be seen in severe heart failure.
3. **Cardiac sclerosis** is a sequela of chronic passive congestion and central hemorrhagic necrosis.
4. **Infarctions** are rare because of the double blood supply (hepatic artery and portal vein).
5. **Hepatic vein thrombosis (Budd-Chiari syndrome)** is a rare syndrome that may be acute or insidious.
   a. **Etiology.** The Budd-Chiari syndrome may be seen in many unrelated conditions, including **neoplasms invading hepatic veins,** polycythemia vera, intrahepatic infection, paroxysmal nocturnal hemoglobinuria, myeloproliferative syndromes, and intravascular webs or membranes. All of these syndromes can provoke clotting either through platelet activation, abnormal platelet function, or activation of the extrinsic clotting system.
6. **Portal vein obstruction and thrombosis**
   a. **Etiology**
      (1) **Extrahepatic causes** include abdominal neoplasms (notably renal cell carcinoma), pancreatitis, sepsis, and postsurgical conditions.
      (2) **Intrahepatic causes** include cirrhosis and primary or secondary neoplastic invasion.
   b. **Clinical features** include portal hypertension and splenomegaly.

E. **Hereditary disorders of bilirubin metabolism**

1. **Gilbert's syndrome** is a benign autosomal dominant disease characterized by **unconjugated hyperbilirubinemia.**
2. **Crigler-Najjar syndrome**
   a. **Type 1** shows autosomal recessive inheritance and complete **absence of glucuronyl transferase,** causing **marked unconjugated hyperbilirubinemia,** severe kernicterus, and death.
   b. **Type 2** shows a mild deficiency of glucuronyl transferase. Kernicterus does not develop.

IN A NUTSHELL

Budd-Chiari syndrome is a hepatic vein obstruction leading to clinical and pathologic features of chronic congested liver.

F. Viral hepatitis

1. **Hepatitis A (HAV)** is a self-limited hepatitis caused by an **RNA virus** with an incubation period of approximately 2-6 weeks. Infection is identified by HAV-specific antibodies (IgM if acute, IgG if past disease). The usual route of infection is **fecal-oral transmission** by contaminated food, particularly mollusks. There is **no carrier state and no chronic disease** (Figure 2-3).
2. **Hepatitis B (HBV)** may cause acute hepatitis, a carrier state, chronic active disease, chronic persistent disease, fulminant hepatitis, or hepatocellular carcinoma. It is caused by a **DNA virus;** the virions are called **Dane particles.** The incubation period is from 1-6 months. Transmission is through contact with infected blood or other body fluids. It can be transmitted by sexual intercourse and is frequently transmitted to newborns of infected mothers by exposure to maternal blood during the birth process.
   a. **Associated antigens** include **core antigen (HBcAg) and surface antigen (HBsAg).** The latter is usually identified in the blood for diagnosis. HbsAg is the earliest marker of acute infection. **HBeAg** is also associated with the core. Its presence indicates active acute infection; when anti-HBeAg appears, the patient is no longer infective (Figure 2-4).
   b. HBV is associated with hepatocellular carcinoma; $HBsAg^+$ patients have a 200-fold greater risk of hepatocellular carcinoma than subjects who have not been exposed.

BRIDGE TO MICROBIOLOGY

- *Hepatitis A is a picornavirus. It has a naked icosahedral nucleocapsid and single-stranded RNA.*
- *Hepatitis B is a double-stranded DNA virus classified as a hepadnavirus. It has an enveloped icosahedral nucleocapsid.*
- *Hepatitis C is classified as a flavivirus. It is a positive-strand RNA virus with an enveloped icosahedral nucleocapsid.*
- *Hepatitis D is caused by the Delta agent—a protein capsule surrounding low-molecular weight RNA.*
- *Hepatitis E is classified as a calicivirus. It is a single-stranded RNA virus with a naked icosahedral nucleocapsid.*

IN A NUTSHELL

- *HBsAg indicates current infection*
- *HBeAg indicates infectivity*

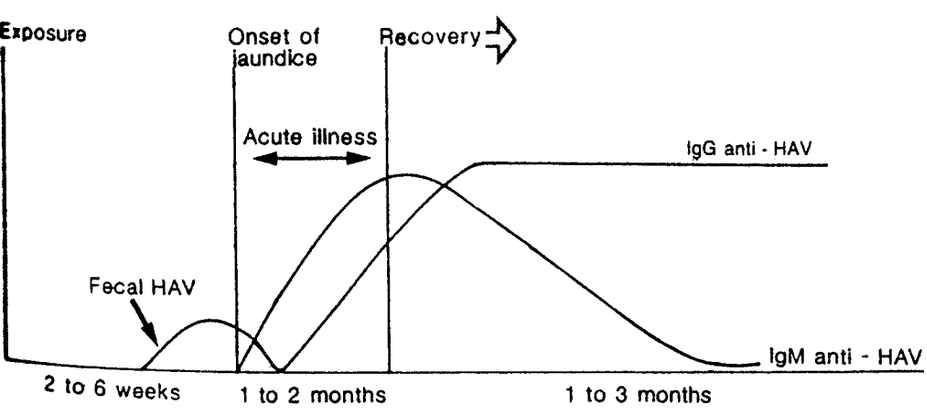


Figure 2-3. Serologic markers in hepatitis A infection.

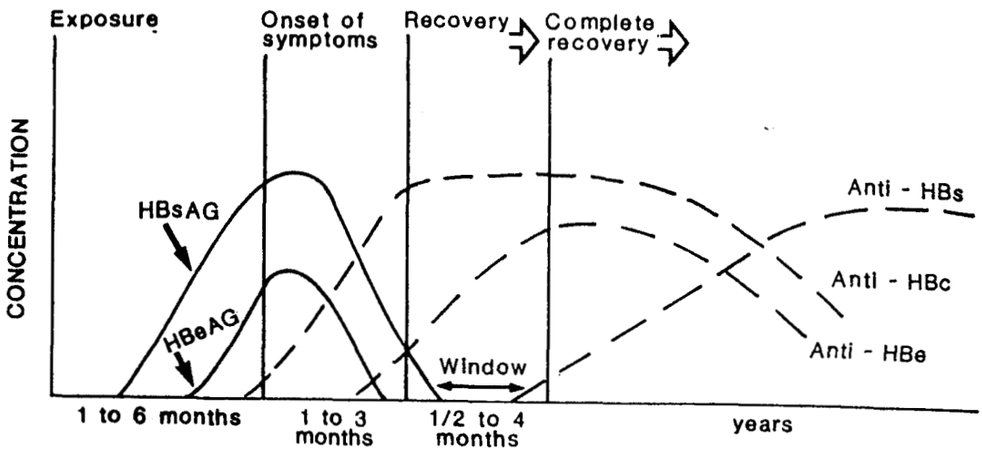


Figure 2-4. Serologic markers in hepatitis B infection.

c. **Antibodies**

(1) **Antibodies to surface antigen (anti-HBs)** are considered protective and usually appear after the disappearance of the virus.

(2) **Antibodies to HBcAg** are not protective. They are detected just after the appearance of HBsAg and are used to confirm infection when both HBsAg and anti-HBs are absent (window).

(3) **Antibodies to HBeAg** are associated with a low risk of infectivity.

3. **Hepatitis C (HCV)** is most often mild and anicteric but occasionally severe with fulminant hepatic failure. It is caused by an RNA virus, which may be transmitted parenterally (a cause of post-transfusion hepatitis); the route of transmission is undetermined in 40%-50% of cases.

   a. Antibody is detected by enzyme-linked immunosorbent assay (ELISA). The incubation period is between 2 and 26 weeks with peak onset of illness 6-8 weeks after infection.

   b. Most patients progress to chronic liver disease, specifically chronic persistent hepatitis or chronic active hepatitis. Cirrhosis is common in patients with chronic active hepatitis and occurs in 20%-25% of infected patients. HCV is also associated with hepatocellular carcinoma.

4. **Delta hepatitis (HDV)** is associated with a 35-nm RNA virus composed of a delta antigen-bearing core surrounded by HBV's Ag coat; **HDV requires HBV for replication.** Delta hepatitis can cause quiescent HBV states to suddenly worsen. Its transmission is the same as that of HBV.

patitis	Mode of transmission	Incubation	Carrier state?	Causes chronic disease?	Genome
patitis A	Fecal-oral	2-6 weeks	No	No	ssRNA
patitis B	Parenteral, sexual	1-6 months	Yes	Yes (5-10% of cases)	DNA
patitis C	Blood transfusion, blood products	2-26 weeks	Yes	Yes (50% of cases)	RNA
lta hepatitis	Parenteral, sexual	1-several months	In association w/hepatitis B	Yes	RNA
patitis E ANB)	Water-borne, fecal-oral	6 weeks	Not known	No	ssRNA

Table 2-5. Types of hepatitis.

5. **Hepatitis E (HEV)** is caused by a single-stranded RNA virus. The disease is typically self-limited and does not evolve into chronic hepatitis; it may, however, be cholestatic. **Pregnant women** may develop fulminant disease. Transmission is by the **fecal-oral** route. HEV occurs mainly in India, Nepal, Pakistan, and Southeast Asia.

6. **Acute viral hepatitis**
   a. **Clinical features.** Acute viral hepatitis may be icteric or anicteric. Symptoms include malaise, anorexia, fever, nausea, upper abdominal pain, and hepatomegaly, followed by jaundice, putty-colored stools, and dark urine. In HBV, patients may have urticaria, arthralgias, arthritis, vasculitis, and glomerulonephritis (because of circulating immune complexes). Blood tests show elevated serum bilirubin (if icteric), elevated transaminases, and alkaline phosphatase. The acute illness usually lasts 4-6 weeks.
   b. **Pathology**
      (1) **Grossly,** there is an enlarged liver with a tense capsule.
      (2) **Microscopically,** there is ballooning degeneration of hepatocytes and liver cell necrosis.

7. **Chronic hepatitis** occurs in 5%-10% of HBV infections and in well over 50% of HCV; it does not occur in HAV. Most chronic disease is due to chronic persistent hepatitis. The chronic form is more likely to occur in the very old or very young, in males, in immunocompromised hosts, in Down's syndrome, and in dialysis patients.
   a. **Chronic persistent hepatitis** is a benign, self-limited disease with a prolonged recovery. Patients are **asymptomatic except for elevated transaminases.**

IN A NUTSHELL

- *HAV, HEV: fecal-oral infections*
- *HBV, HCV, HDV: parenteral infections*

IN A NUTSHELL

Pathology of hepatitis:

Grossly, enlarged liver; microscopically, coagulative necrosis with increased eosinophilia.

NOTE

HBV and HCV can lead to chronic hepatitis, and may predispose to hepatocellular carcinoma.

b. **Chronic active hepatitis** features chronic inflammation with hepatocyte destruction, resulting in cirrhosis and liver failure.
   (1) **Etiology**. HBV, HCV, HDV, drug toxicity, Wilson's disease, alcohol, α_1-antitrypsin deficiency, and autoimmune hepatitis are common etiologies.
   (2) **Clinical features** may include fatigue, fever, malaise, anorexia, and elevated liver function tests.
   (3) **Diagnosis** is made by liver biopsy.

8. **Carrier state** for HBV and HCV may be either asymptomatic or with liver disease; in the latter case, the patient has elevated transaminases.
   a. **Incidence** is most common in immunodeficient, drug addicted, Down's syndrome, and dialysis patients.
   b. **Pathology** of asymptomatic carriers shows **"ground-glass" hepatocytes** with finely granular eosinophilic cytoplasm.

9. **Fulminant hepatitis** leads to submassive and massive hepatic necrosis.
   a. **Etiology.** HAV, HBV, HCV, delta virus (HDV) superinfection, HEV, chloroform, carbon tetrachloride, isoniazid, halothane, and other drugs (acetaminophen overdose) all may cause fulminant hepatitis.
   b. **Clinical features** include progressive hepatic dysfunction with a mortality of 25%-90%.
   c. **Pathology**
      (1) **Grossly,** one sees progressive shrinkage of the liver as the parenchyma is destroyed.

CLINICAL CORRELATE

Infants with HBV infected postnatally or during birth rarely develop active hepatitis but they often become chronic carriers. They also have an increased rate for hepatocellular carcinoma and hepatic cirrhosis.

G. **Cholangitis is inflammation of the bile ducts.**
   1. It is usually associated with biliary duct obstruction by gallstones or carcinoma, which leads to infection with enteric organisms. This results in purulent exudation within the bile ducts and bile stasis.
   2. Clinically, cholangitis presents with jaundice, fever, chills, leukocytosis, and right upper quadrant pain.

CLINICAL CORRELATE

Cholangitis = Charcot's triad = jaundice, fever, and right upper quadrant pain.

H. **Pericholangitis** is inflammation around the bile ducts without intraductal involvement.

I. **Pyogenic liver abscesses** may be caused by *E. coli,* Klebsiella, Streptococcus, Staphylococcus, Bacteroides, Pseudomonas, and fungi.

J. **Parasitic infections**
   1. **Schistosomiasis** is caused by different organisms in different parts of the world.

a. **Clinical features** include splenomegaly, portal hypertension, and ascites. Lesions are caused by the immune response to ova.

2. **Amebiasis** is caused by *Entamoeba histolytica.*
   a. **Clinical features** include bloody diarrhea, pain, fever, jaundice, and hepatomegaly.

K. **Drug-induced liver damage** may be caused by agents that are direct hepatotoxins, such as carbon tetrachloride, acetaminophen, methotrexate, anabolic steroids, and oral contraceptive pills.

L. **Cirrhosis** is the diffuse involvement of the whole liver by fibrosis due to hepatocellular injury, fibrosis in the form of dense scars or delicate bands, and nodules caused by fibrous bands and regenerating hepatocytes. These so called **regenerative nodules**, which lack the usual architectural landmarks such as ordered sinusoids and a central vein, are hallmarks of cirrhosis.

1. **Epidemiology.** Cirrhosis is the third leading cause of death in the 25-65-year-old age group. Leading types include alcoholic cirrhosis, postnecrotic cirrhosis, biliary cirrhosis, and hemochromatosis-related cirrhosis.

2. **Clinical features**
   a. **Portal hypertension** is most commonly caused by cirrhosis of the liver.
      (1) **Other causes** include posthepatic (e.g., right-sided heart failure, Budd-Chiari syndrome), prehepatic (e.g., portal vein obstruction), or intrahepatic (e.g., schistosomiasis, sarcoid).
      (2) **Signs and symptoms** include **ascites** (an accumulation of fluid in the peritoneal cavity); portosystemic shunts that form **hemorrhoids, esophageal varices** (which may cause massive hematemesis), periumbilical **(caput medusae),** and retroperitoneal dilatations, and portosystemic encephalopathy; and **splenomegaly** with hypersplenism.
   b. **Impaired estrogen metabolism** and male hypogonadism may cause female hair distribution and **gynecomastia** in males, gonadal atrophy, amenorrhea in females, **spider angiomata,** and **palmar erythema.**
   c. Other associated disorders include **Dupuytren's contractures,** hypoalbuminemia, peripheral edema, low levels of vitamin K-dependent clotting factors (causing **bleeding diathesis**), rare hepatorenal syndromes, and hepatic encephalopathy.

NOTE

Schistosomes, Echinococcus, and Entamoeba are three parasitic infections of the liver.

IN A NUTSHELL

Cirrhosis is the diffuse fibrosis and regeneration of the liver due to hepatocellular injury by toxins, drugs, viruses, or deposition of metabolites or minerals (e.g., glycogen storage diseases; Wilson's disease).

CLINICAL CORRELATE

Cirrhosis and portal hypertension can cause numerous physical exam findings:

- *Ascites (↓ albumin synthesis)*
- *Varices, hemorrhoids, and caput medusae (due to portosystemic shunts)*
- *Splenomegaly*
- *Gynecomastia, spider angioma, and palmar erythema (due to impaired estrogen metabolism)*
- *Dupuytren's contractures and clubbing*
- *Bleeding diathesis*

CLINICAL CORRELATE

The prothrombin time (PT), not the PTT, is used to assess coagulopathy due to liver disease.

3. **Etiologies.** Already mentioned are the chronic hepatidites (HBV, HCV, but never HAV, HEV) and chronic drug reactions.
   a. **Postnecrotic cirrhosis** produces a macronodular pattern.
      (1) **Etiology.** Most cases are secondary to chronic active hepatitis.
   b. **Biliary cirrhosis**
      (1) **Primary biliary cirrhosis** has an **autoimmune etiology** and causes sclerosing cholangitis and cholangiolitis. It is associated with other autoimmune diseases and primarily affects **middle-aged women.**
      (2) **Secondary biliary cirrhosis** is caused by long-standing large bile duct obstruction, producing stasis of bile, leading to inflammation, secondary infection, and scarring. It usually presents with jaundice.
      (3) **Sclerosing cholangitis** is a chronic fibrosing inflammatory disease of the extrahepatic and larger intrahepatic bile ducts.
   c. **Hemochromatosis** is a disease with autosomal recessive inheritance. **Deposits of iron** occur in the liver, pancreas, heart, adrenal, thyroid, parathyroid, and anterior pituitary with resultant organ dysfunction. It should be distinguished from **hemosiderosis,** which is a term used to describe iron overload from any cause.
   d. **Wilson's disease (hepatolenticular degeneration)** is an autosomal recessive disease characterized by **inadequate hepatic excretion of copper.** Wilson's disease causes hepatitis or macronodular cirrhosis, degenerative changes in the lenticular nuclei of the brain, and pathognomonic **Kayser-Fleischer rings,** a deposition of copper in the corneal limbus.
   e. **Alpha$_1$-antitrypsin deficiency** is an autosomal recessive disease characterized by deficiency of a protease inhibitor, resulting in **pulmonary emphysema** and **hepatic damage.**
   f. **Syphilitic cirrhosis** causes scarring due to gummas.

CLINICAL CORRELATE

Hemosiderin deposits are seen in the liver and pancreas of hemochromatosis patients.

CLINICAL CORRELATE

Both hemochromatosis and Wilson's disease are associated with an increased risk of hepatocellular carcinoma.

M. **Alcoholic liver disease** causes fatty liver, alcoholic hepatitis, and alcoholic cirrhosis, which are separate though possibly interrelated entities.

1. **Epidemiology.** Alcoholic liver disease accounts for 60%-70% of cirrhosis in the Western Hemisphere. The male:female ratio is 2:1. There is a possible genetic predisposition.
2. **Clinical features.** Fatty change is generally asymptomatic. Alcoholic hepatitis presents with fever; hepatomegaly; jaundice; elevated aspartate transaminase (AST), alkaline phosphatase, and alanine aminotransferase (ALT); and possible portal hypertension. Cirrhosis often presents with portal hypertension. Patients die due to liver failure, infection, upper gastrointestinal bleeds, hepatocellular carcinoma, encephalopathy, and renal failure (secondary to hepatorenal syndrome).
3. **Pathology**
   a. **Fatty liver (steatosis)** is reversible.
      (1) **Grossly,** fatty changes appear as a yellow, greasy, enlarged liver.
   b. **Alcoholic hepatitis** is usually associated with fatty change and is occasionally seen with cirrhosis. It results from prolonged alcoholic abuse. Pathologic findings include swelling of hepatocytes, followed by necrosis and polymorphonuclear inflammation, formation of alcoholic hyaline **(Mallory bodies)** in swollen hepatocytes, cholestasis, and beginning fibrosis. The appearance of fibrosis may be linked to the onset of cirrhosis.
   c. **Alcoholic cirrhosis.** Early stages of disease show a large liver with micronodular formation. The end stage resembles postnecrotic cirrhosis. The amount of fat decreases as the amount of fibrous tissue increases.

N. **Benign tumors**

1. **Liver cell adenoma** incidence is increased with anabolic steroid and oral contraceptive use. It forms a mass, which may be mistaken for carcinoma or may rupture (especially during pregnancy).
2. **Nodular hyperplasia**
   a. **Focal nodular hyperplasia** refers to a solitary nodule that often has a fibrous capsule and bile ductules.
   b. **Nodular regenerative hyperplasia** describes multiple nodules composed of normal hepatocytes with a loss of normal radial architecture.

IN A NUTSHELL

Alcoholic liver disease:

- *Fatty liver*
- *Alcoholic hepatitis*
- *Alcoholic cirrhosis*

NOTE

Steatosis is usually asymptomatic and reversible. Fatty vacuoles displace hepatocellular nuclei peripherally.

NOTE

Mallory bodies may also be seen in Wilson's disease, hepatocellular carcinoma, and primary biliary cirrhosis. Besides history, the other helpful feature in distinguishing alcoholic hepatitis from these other entities is the extreme fatty change.

CLINICAL CORRELATE

In addition to liver disease, alcoholics suffer from a variety of other disorders. If a question presents an alcoholic patient, keep in mind increased incidence of the following disorders:

Esophagus:
- *Cancer*
- *Mallory-Weiss tears (after vomiting)*
- *Varices (with portal hypertension)*

Stomach:
- *Gastritis, reflux*
- *Peptic ulcer disease*

Pancreas:
- *Pancreatitis (#1 cause of chronic pancreatitis)*
- *Cancer*

Cardiac:
- *Cardiomyopathy (dilated)*

Respiratory:
- *Aspiration pneumonia*
- *Klebsiella pneumonia*
- *Tuberculosis*

Heme:
- *Megaloblastic anemia (folate deficiency)*
- *Coagulation defects (liver dysfunction)*
- *Thrombocytopenia due to congestive splenomegaly*
- *Acquired sideroblastic anemia*

Neuromuscular:
- *Wernicke's encephalopathy*
- *Korsakoff's amnestic syndrome*
- *Vestibulopathy*
- *Peripheral neuropathies*
- *Acute cerebellar degeneration*
- *Myopathy (in chronic alcoholism)*
- *Alcohol withdrawal syndrome; delirium tremens*

Acid/Base:
- *Ketoacidosis (increased anion gap)*

IN A NUTSHELL

Hepatocellular carcinoma is predisposed by cirrhosis, HBV, oral contraceptives, and aflatoxin B (fungal toxin).

3. **Cavernous hemangiomas** are large, vascular, endothelial-lined spaces filled with red cells. Radiologically, they must be considered in the differential diagnosis of metastases to the liver.
4. **Bile duct adenomas** form small nodules that are not bile stained.
5. **Cysts** may be single (with serous fluid) or multiple (with brown, bile-stained fluid). Multiple cysts may be associated with adult polycystic kidney disease.

O. **Malignant tumors**

1. **Hepatocellular carcinoma (hepatoma)**
   a. **Epidemiology.** Hepatocellular carcinoma comprises 90% of primary liver neoplasms. It is strongly associated with cirrhosis and HBV infection, as well as with oral contraceptives, androgens, and aflatoxin B.
   b. **Clinical features** include tender hepatomegaly, ascites, weight loss, fever, polycythemia, and hypoglycemia. A friction rub may also be heard. **Alpha-fetoprotein (AFP) is** present in 50%-90% of patients' serum. (AFP is also found with other forms of liver disease, pregnancy, fetal neural tube defects, and germ cell carcinomas of the ovaries and testes.) Death is due to gastrointestinal bleed and liver failure. Generally, metastases first occur in the lungs.
2. **Cholangiocarcinomas** are cancers of the intrahepatic bile duct and comprise 10% of primary liver neoplasms. In developing countries, this tumor is associated with infection with ***Clonorchis sinensis*** (liver fluke) and with primary sclerosing cholangitis.
3. **Hepatoblastoma** is a rare, malignant neoplasm of children.
4. **Angiosarcoma** is associated with exposure to vinyl chloride and arsenic.
5. **Metastic tumors** are much more common than primary neoplasms, most commonly coming from the breast, lung, and colon. Multiple, well-circumscribed nodules in a markedly enlarged liver are seen (Figure 2-5).

Figure 2-5. Pancreatic carcinoma metastatic to liver (gross).

P. **Reye's syndrome** is characterized by **fatty change** in the liver and edematous **encephalopathy**. It usually affects children between 6 months and 15 years of age.

1. **Etiology** is unclear. It is frequently preceded by a mild upper respiratory infection, varicella, or influenza A or B infection. It is also associated with **aspirin administration** at levels that are not ordinarily toxic.

BILIARY DISEASE

A. **Cholelithiasis (gallstones)**

1. **Incidence.** Cholelithiasis occurs in 20% of women and 8% of men in the United States. It is rare before age 20, but is seen in 25% of persons greater than 60 years old.
2. **Etiology**
   a. **Cholesterol stones**
      (1) **Pure cholesterol stones** are radiolucent, solitary, 1-5 cm in diameter, yellow, and smooth, with a glistening radial pattern on cut section.
      (2) The typical patient is **fat, female, fertile** (multiparous), and **over forty** years of age (the "4-F's").
      (3) Exogenous estrogens, clofibrate, high-calorie diet, obesity, diabetes mellitus, pregnancy, celiac disease, and increasing age all predispose to cholesterol stones.

IN A NUTSHELL

Reye's syndrome:

- *Fatty liver changes*
- *Vomiting*
- *Encephalopathy*
- *Preceded by URI or chickenpox with aspirin administration*

b. **Pigment stones** are small, black, multiple, and radiolucent. Pigment stones are clumps of pigment derived from **unconjugated bilirubin.**

c. **Mixed stones** comprise 80% of all stones and are associated with chronic cholecystitis. They are composed of cholesterol and calcium bilirubinate.

3. **Pathogenesis.** Supersaturation of bile pigment or cholesterol and/or a decreased amount of phospholipid or bile salts predisposes to stone formation.

4. **Clinical features**

a. Most stones remain in the gallbladder and are asymptomatic.

b. Obstruction of the gallbladder or cystic duct may cause biliary colic, acute cholecystitis, or hydrops (mucocele of the gallbladder).

c. Obstruction of the common bile duct may lead to obstructive jaundice, and ascending cholangitis. Pancreatitis and gallstone ileus may also result from blockage of the ampulla of Vater or distal small bowel, respectively.

B. **Acute cholecystitis.** Most cases are caused by obstruction of the neck of the gallbladder or cystic duct by gallstones.

CLINICAL CORRELATE

When cholelithiasis occurs in a young person, think of hereditary spherocytosis, sickle cell disease, or other chronic hemolytic process.

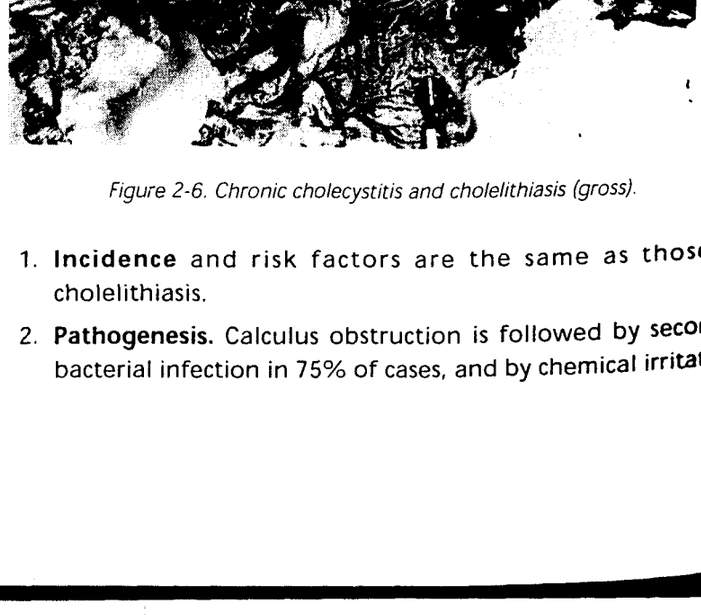


Figure 2-6. Chronic cholecystitis and cholelithiasis (gross).

NOTE

Cholecystitis (right upper quadrant pain, fever, leukocytosis) may result from superinfection of cholelithiasis.

1. **Incidence** and risk factors are the same as those for cholelithiasis.

2. **Pathogenesis.** Calculus obstruction is followed by secondary bacterial infection in 75% of cases, and by chemical irritation.

3. **Clinical features**
   a. Acute cholecystitis presents with acute onset of **right upper quadrant pain,** fever, tenderness, and leukocytosis.
   b. Most cases resolve with medical management. The remainder progress to empyema, gangrenous necrosis, or rupture. Patients exhibit symptoms of acute abdomen and require cholecystectomy.

C. **Chronic cholecystitis** is usually not preceded by acute cholecystitis but is always accompanied by cholelithiasis.

1. **Pathogenesis** is unclear. Inflammation is probably due to chemical injury from supersaturated bile, not to irritation by stones.

D. **Cholesterolosis** refers to lipid foci deposited in the gallbladder wall ("strawberry" gallbladder). It is asymptomatic and unrelated to cholelithiasis.

E. **Benign tumors**

1. **Papillomas** are small, pedunculated, branching lesions.
2. **Adenomas** form small, flat, elevated plaques.
3. **Adenomyomas** are a proliferation of smooth muscle and glands.

F. **Malignant tumors**

1. **Carcinoma of gallbladder**
   a. **Incidence.** The disease occurs predominantly in the elderly.
   b. **Risk factors** include cholelithiasis and cholecystitis (in up to 90% of patients).
   c. **Clinical features.** The disease is asymptomatic until late. It may present with dull abdominal pain, mass, weight loss, and anorexia.
   d. **Pathology**
      (1) **Grossly,** the tumor typically involves the fundus and neck.
      (2) **Microscopically,** 90% are differentiated or undifferentiated adenocarcinomas.
   e. **Prognosis** is poor, with a 3% five-year survival rate.
2. **Carcinoma of bile ducts (cholangiocarcinoma)**
   a. **Incidence.** Men are affected more frequently than women, and patients are usually elderly.
   b. **Risk factors** include chronic inflammation, infections, (e.g., liver flukes), and ulcerative colitis.
   c. **Clinical features.** The disease presents with obstructive jaundice and its associated symptomatology.
   d. **Prognosis** is usually poor because of ductal, lymphatic, and, to a lesser extent, hematogenous spread.

IN A NUTSHELL

Gallbladder cancer is rare, often asymptomatic, and is usually adenocarcinoma.

CLINICAL CORRELATE

Cholangiocarcinoma (cancer of the bile ducts) often presents with obstructive jaundice, right upper quadrant pain, and sometimes symptoms of pancreatitis due to obstruction of the pancreatic duct.

NUTRITIONAL DISORDERS

A. **Marasmus**

1. **Definition.** Marasmus is a condition of severe malnutrition or emaciation resulting from **inadequate calorie intake.**
2. **Clinical features** include "failure to thrive," loss of subcutaneous fat, muscle wasting, and a lower percentile in weight than in height.
3. **Pathology.** There is generalized hypoplasia and atrophy of tissues, and there may be mild anemia.

B. **Kwashiorkor**

1. **Definition.** Kwashiorkor refers specifically to **inadequate protein intake.** It may develop despite adequate caloric intake.
2. **Clinical features** include edema, anemia, dermatoses (e.g., pigmentary changes, desquamation, dusky erythema), hepatomegaly, hair changes, growth retardation, irritability, apathy, and low energy.

IN A NUTSHELL

Marasmus:
- *Calorie deficiency*
- *No edema*

Kwashiorkor:
- *Protein deficiency*
- *Edema*

3. **Laboratory values.** Serum chemistry shows decreased serum total protein and albumin.

Iron deficiency

1. **Etiology.** Although iron deficiency is caused by dietary insufficiency, malabsorption may also play a causative role. In the United States, the most common cause of iron deficiency is blood loss from the GI tract.
2. **Clinical features** of iron deficiency, all of which are due to the varied metabolic functions of iron, include:
   a. **Hypochromic microcytic anemia**
   b. Functional folate deficiency
   c. Depressed cell-mediated immunity
   d. Gastric erosions

Zinc deficiency

1. **Etiology** is usually dietary insufficiency.
2. **Clinical features** include delayed wound healing, short stature, and diminished axillary, facial, and pubic hair. Zinc deficiency may predispose to alcoholic cirrhosis.

Cardiovascular Pathology

Cardiovascular disease affects a large segment of the population. In fact, atherosclerosis is the leading cause of death in the United States. Because the effects of cardiovascular disease are widespread and potentially lethal, it is important to be able to identify the major risk factors for each type of disease. This chapter will focus on the structural and functional changes that occur with diseases of the heart and vessels, as well as the major risk factors associated with each disease entity. Note that conduction abnormalities and arrhythmias were reviewed in the Cardiovascular Physiology section.

CONGENITAL ABNORMALITIES OF THE HEART

A. **Overview**

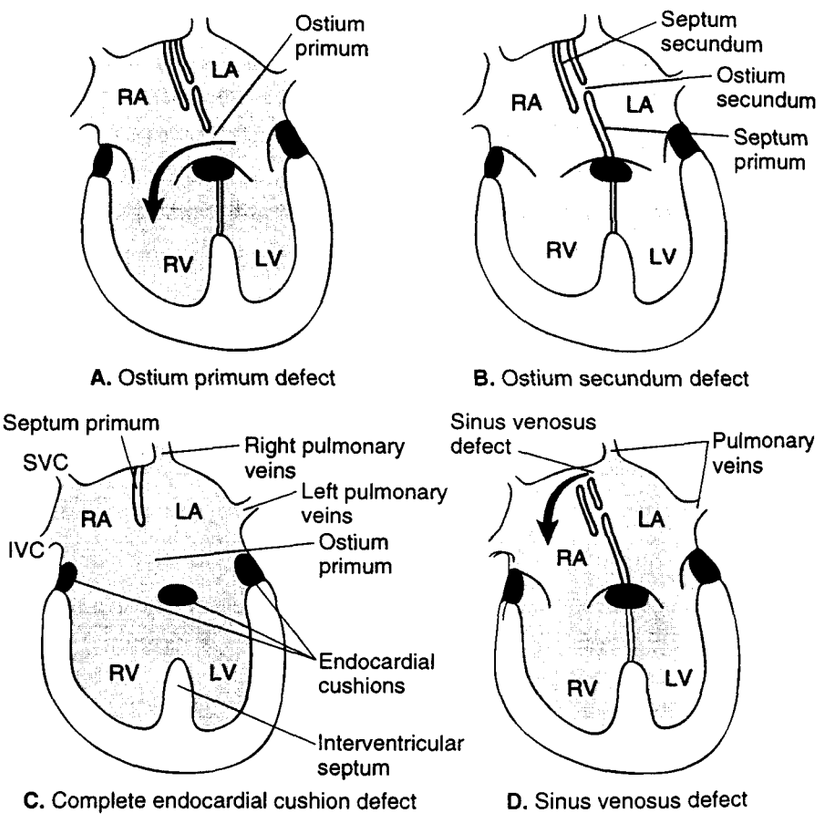
1. **Etiology**
   a. During cardiac development, insults must occur before the end of week 16 (completion of heart development) in order for a congenital defect to occur.
   b. Chromosomal abnormalities (e.g., trisomy 13, 18, 21) may lead to specific cardiac anomalies.
   c. Mendelian hereditary syndromes (e.g., Marfan's, Ehlers-Danlos) may also be associated with specific cardiac defects as well as with other developmental anomalies .
   d. Environmental causes (e.g., maternal rubella, alcohol, smoking) may have variable effects, depending on when and how severe the insult is to the mother and fetus.
   e. Up to 90% of congenital heart disease is of unknown etiology.
   f. Cardiomegaly, heart murmurs, and congestive heart failure
   g. Chronic cyanosis, which will cause polycythemia, clubbing of fingers and toes, and hypertrophic osteoarthropathy

B. **Acyanotic congenital heart disease** (left-to-right shunts). In this disease, blood is abnormally shunted from the **left to the right** side of the heart. This causes chronic right heart failure and secondary pulmonary hypertension as a result of increased pressure and flow. Right heart pressure may eventually increase to become greater than left heart pressure, and the shunt will reverse, becoming a right-to-left shunt that results in late onset cyanosis.

1. **Ventricular septal defect (VSD)** is an abnormal communication between ventricles, usually at the membranous interventricular septum. The clinical significance depends on the volume of the shunt. It is often associated with other defects.

CLINICAL CORRELATE

Some of the loudest murmurs are VSDs.



A. Ostium primum defect

B. Ostium secundum defect

C. Complete endocardial cushion defect

D. Sinus venosus defect

Figure 3-1. Common configurations of atrial septal defects.

NOTE

In approximately 25% of adult hearts, the atrial septum actually remains open, but is kept functionally closed by the normal pressure differential between the right and left atria.

2. **Atrial septal defect (ASD)** is an abnormal communication between the atria (Figure 3-1). The clinical significance depends on the volume of the shunt.
   a. **Ostium primum defects** account for approximately 5% of all ASDs. The defect is in the lower atrial septum above the atrioventricular (AV) valves. It may be associated with maldeveloped AV valves.

b. **Ostium secundum** defects account for approximately 90% of all ASDs. The defect is in the center of the atrial septum at the foramen ovale, resulting from abnormalities of the septum primum, septum secundum, or both. It is not associated with maldeveloped AV valves.
c. **Complete endocardial cushion defect** results in an ASD, a VSD, and a common AV canal.
d. **Sinus venosus** accounts for approximately 5% of all ASDs. It causes a defect in the upper part of the atrial septum and may cause anomalous venous return from the pulmonary veins into the superior vena cava or right atrium.
e. **Patent foramen ovale** is a slit-like remnant of the foramen ovale and is usually not of clinical significance.

3. **Patent ductus arteriosus (PDA)** is a defect in which oxygenated blood flows from the aorta to the pulmonary artery. This deprives the systemic circulation of oxygenated blood and eventually leads to pulmonary hypertension. Indomethacin can be used to close a PDA, while the prostaglandin PGE can be utilized to keep it open if necessary.

NOTE

PDA causes pulmonary hypertension due to excess blood to pulmonary artery (leads to ↑ pressure).

C. **Cyanotic congenital heart disease** (right-to-left shunts). In these diseases, blood is shunted from the **right to the left** side of the heart, causing poorly oxygenated blood to be pumped out to the systemic circulation. This causes immediate cyanosis and permits paradoxic embolism, in which venous emboli bypass the pulmonary circulation and directly enter the systemic circulation (Figure 3-2).

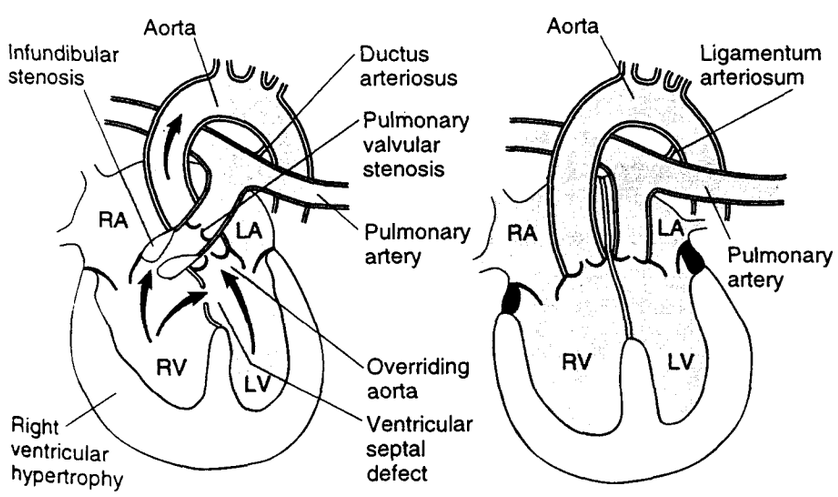


Figure 3-2. Common forms of cyanotic congenital heart disease.

1. **Tetralogy of Fallot** has four specific anomalies and is the most common cyanotic congenital heart disease in older children and adults.
   a. The four lesions are:
      (1) **VSD**
      (2) **An overriding aorta** that receives blood from both ventricles
      (3) **Right ventricular hypertrophy**
      (4) **Pulmonic stenosis** (right ventricular outflow obstruction)
   b. The clinical significance depends on the degree of right ventricular outflow obstruction.
   c. Deoxygenated blood is shunted to the left side of the heart through the VSD, and blood flows from both ventricles into the enlarged aorta with little reaching the lungs.
   d. A PDA permits survival if the pulmonary artery is completely obstructed.
2. **Transposition of the great vessels** results from a failure of the truncoconal septum to spiral during development. The aorta arises from the right ventricle, and the pulmonary artery arises from the left ventricle. This is often associated with other anomalies. Either PDA, a VSD, an ASD, or a patent foramen ovale mixes venous and systemic blood and, therefore, permits survival.
3. **Persistent truncus arteriosus** is the failure of the aorta and pulmonary arteries to separate. There is also a VSD, usually at the membranous interventricular septum. The truncus arteriosus receives blood from both ventricles, so cyanosis results.

D. **Obstructive congenital heart disease.** This form of developmental defect typically does not cause cyanosis.

1. **Coarctation of the aorta**
   a. In the **preductal (infantile) type,** there is narrowing of the aorta proximal to the opening of the ductus arteriosus.
   b. In the **postductal (adult) type,** there is narrowing of the aorta distal to the opening of the ductus arteriosus. This is the most common type, and generally allows survival into adulthood.
2. **Pulmonary valve stenosis or atresia.** This lesion may be due to an unequal division of the truncus arteriosus so that the pulmonary trunk has no lumen or opening at the level of the pulmonary valve. It may cause cyanosis if severe.
3. **Aortic valve stenosis or atresia**
   a. Complete atresia will not support neonatal life.
   b. Bicuspid aortic valves may be asymptomatic but can lead to infective endocarditis, left ventricular overload, and sudden

death. This lesion is the second most common cause of aortic stenosis, after rheumatic fever.

E. **Abnormalities associated with genetic syndromes**

1. **Marfan's syndrome.** One-third of patients have congenital cardiovascular disease characterized by aortic dilatation and incompetence, aortic dissection, and ASD.
2. **Down's syndrome** (trisomy 21). Twenty percent of patients may have congenital cardiovascular disease, characterized by an ostium primum type of ASD and a VSD.
3. **Turner's syndrome** patients frequently have coarctation of the aorta and pulmonary stenosis.

F. **Abnormalities associated with perinatal insults**

1. **Maternal infection with rubella** in the fifth to tenth weeks can lead to PDA, ASD, and VSD.
2. **Fetal alcohol syndrome** can lead to cardiovascular defects, including VSD.
3. **Maternal ingestion of drugs** such as trimethadione (anti-seizure medication) and isotretionin (retin-A) can cause fetal cardiac defects.

ISCHEMIC HEART DISEASE (IHD)

A. **Overview**

1. IHD occurs when the oxygen supply does not meet the oxygen demand of the myocardial tissue. It is the leading cause of death (along with hypertensive and valvular diseases) in the United States.
2. Ischemia is caused (alone or in combination) by the following conditions:
   a. **Narrowing of the coronary arteries,** often precipitated by vasospasm or overlying thrombus is the most frequent cause of cardiac ischemia. Most infarctions occur when multiple vessels are narrowed.
      (1) Ninety percent of cases are due to atherosclerosis.
      (2) The underlying lesion is usually a complicated plaque, with calcification, ulceration, or overlying thrombus.
      (3) Less common causes include dissecting aortic aneurysm, arteritis, coronary embolism, and cocaine-induced vasospasm.
      (4) Perfusion is impaired when the cross-sectional area of the lumen is reduced by more than 75%.

NOTE

The pathophysiology of atherosclerosis is discussed later on in this chapter.

b. **Decreased oxygen-carrying capacity** of the blood from anemia, carbon monoxide poisoning, pulmonary disease, or smoking may lead to cardiac ischemia, particularly in combination with atherosclerosis.
c. **Increased myocardial demand** from tachycardia or hypertrophy may also increase the risk of ischemia.

3. IHD is categorized into four syndromes.
   a. **Angina pectoris** is pain due to ischemia. Patients with angina have an increased incidence of myocardial infarction.
   b. **Myocardial infarction** is ischemic necrosis due to insufficient blood supply.
   c. **Chronic ischemic heart disease** may or may not cause angina or myocardial infarction. Some patients merely experience heart failure.
   d. **Sudden cardiac death** is the presenting symptom in 25% of patients with IHD.

B. **Angina pectoris.** This syndrome is **paroxysmal substernal or precordial chest pain,** caused by transient myocardial ischemia *without* myocardial infarction. Prolonged and repeated angina pectoris may cause focal fibrosis and subendocardial myocardial vacuolization, indicating gradual loss of myocytes. Angina and its resultant fibrosis are associated with impaired diastolic relaxation, increased diastolic filling pressure, and subsequent pulmonary congestion with resultant dyspnea.

1. **Stable angina pectoris**
   a. Paroxysms are associated with a fixed amount of exertion.
   b. Typical attacks last less than 10 minutes and are relieved with rest or sublingual nitroglycerin.
   c. EKG may show ST segment depressions (ischemia limited to subendocardium).
   d. Most angina pectoris is caused by severe atherosclerotic narrowing of coronary arteries.

2. **Prinzmetal's angina pectoris** (paroxysmal vasospasm)
   a. Vasospasm causes decreased blood flow through atherosclerotic vessels.
   b. This form of attack frequently occurs at rest with ST-segment elevations on EKG.

3. **Unstable angina pectoris** often leads to myocardial infarction.
   a. It can present with prolonged chest pain, abnormally severe pain, or pain at rest in a person with stable angina.
   b. It is often unresponsive to nitroglycerin.

C. Myocardial infarction

1. Overview

a. Myocardial infarction is **ischemic necrosis** of the myocardium, resulting from an abrupt decrease in coronary blood flow or a sudden demand for increased myocardial delivery, which cannot be met because of coronary artery narrowing. It is more commonly transmural, but can be subendocardial.

b. Myocardial infarction is more common in men than women; the highest incidence of fatal myocardial infarction is from 55-64 years old.

c. **Risk factors** include hypertension, hypercholesterolemia, cigarette smoking, family history, diabetes mellitus, oral contraceptive use, and sedentary life style.

d. Type A personalities (aggressive, compulsive) may have an increased risk.

e. **Regular exercise and moderate alcohol use** (one glass of wine per day) may decrease the risk by raising the level of high-density lipoprotein (HDL).

2. Types of infarcts

a. **Transmural infarcts** are infarctions of the full thickness of the ventricular wall. They are usually due to occlusion of vessels from severe coronary atherosclerosis, formation of complicated atheromatous plaques with ulceration or fissure, and thrombosis. Occlusions occur most often in the proximal left anterior descending (50%), right coronary (35%), and left circumflex (15%) arteries.

b. **Subendocardial infarcts** are infarctions limited to the inner half of the ventricular wall. The subendocardium is more vulnerable to generalized myocardial hypoperfusion than are other areas, usually because of diffuse atherosclerosis without thrombosis and resultant borderline ischemia. Infarction occurs when flow is further compromised (e.g., CHF, shock, arrhythmia, vasospasm, severe hypertension) or when oxygen demand is increased (e.g., exertion, tachycardia).

4. Clinical features

a. There is acute, severe, crushing chest pain, often radiating to the jaw or left arm.

b. The pain is associated with diaphoresis, a sense of impending doom, nausea, anxiety, and shortness of breath.

c. ECG abnormalities consist of ST elevation and T-wave inversion (with Q-wave development) and ST depression (without Q-wave development). Q waves may develop with transmural infarcts.

NOTE

Atypical presentations of MI with little or no chest pain can be seen most frequently in women, the elderly, diabetic patients, and surgical patients.

d. Elevated cardiac enzymes (Figure 3-3) include:
   (1) **MB isoenzyme of creatine kinase (CK-MB),** which is the most sensitive in common use
   (2) **Lactate dehydrogenase** (specifically the LDH 1 isoenzyme), which is elevated rather specifically in myocardial infarction
   (3) **Serum glutamic-oxaloacetic transaminase** (SGOT) or **aspartate transaminase** (AST), also rises and falls predictably in myocardial infarction, but may indicate liver damage instead.

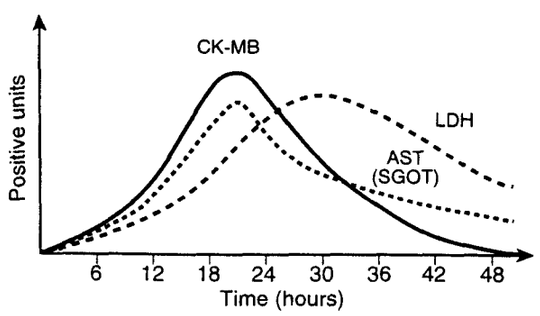


Figure 3-3. Enzymology of myocardial infarction. CK = creatine kinase; LDH = lactate dehydrogenase; AST = aspartate transaminase; and SGOT = serum glutamic-oxaloacetic transaminase.

5. **Prognosis**
   a. Sudden cardiac death, secondary to a fatal arrhythmia, occurs in 25% of patients with an acute myocardial infarction.
   b. Mortality after myocardial infarction is 35% in the first year, 45% in the second year, and 55% in the third year.
   c. 85–90% of survivors develop complications.
6. **Complications** are listed below in order of frequency.
   a. **Arrhythmias** (ventricular fibrillation is the most serious). Ischemia and necrosis of the AV node and three fascicles of the conduction system can lead to heart block with compromise of cardiac function.
   b. **CHF** may be seen with a loss of 20% or more of the ventricular muscle.
   c. **Cardiogenic shock** results when there is a loss of 40% or more of the left ventricular muscle, resulting in the inability of the heart to maintain an adequate output to vital organs. Mortality is greater than 80%.
   d. **Thrombus formation,** resulting from lack of contractility of the infarcted area and abnormal endothelium, may follow

myocardial infarction. This may be a source of systemic or cerebral emboli in large anterior wall infarctions.

e. **Aneurysm formation** (outpouching of noncontractile scar) results in depression of cardiac output; rupture is uncommon. It may be the site of ectopic ventricular electrical activity, leading to fibrillation.

f. **Myocardial (ventricular) rupture**

(1) Patients are most susceptible 1-7 days after infarct, when the myocardium is necrotic but granulation tissue formation has not really begun.

(2) Rupture is most common with anteroapical infarction and is almost uniformly fatal; it results from sudden bleeding into the pericardial sac and tamponade.

g. **Postinfarction pericarditis** (Dressler's syndrome) occurs 2-10 weeks postinfarction.

h. **Acute mitral insufficiency** may be caused by papillary muscle infarction with or without rupture.

7. **Treatment and management**

a. **Coronary artery bypass** with saphenous vein or internal mammary artery grafts restores circulation and eliminates angina. Grafts last approximately 10 years before restenosis typically occurs.

b. **Angioplasty** (balloon dilatation) also restores circulation; half restenose in 1 year.

RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE

A. **Acute rheumatic fever (ARF)** is a recurrent inflammatory disease that typically follows pharyngitis caused by **group A β-hemolytic streptococci.**

1. **Incidence.** ARF is found mainly in children 5-15 years old. There has been a declining incidence and mortality in the last 40 years, mostly due to penicillin, though the incidence began to drop even before penicillin was widely used.

2. **Pathogenesis.** Antistreptococcal antibodies made by the infected host cross-react with host connective tissue (i.e., cardiac, pulmonary, synovial, peritoneal) antigens and lead to end-organ damage by an immunologic mechanism.

3. **Clinical features**

a. Onset is typically 1-3 weeks after streptococcal pharyngitis, otitis media, or tonsillitis.

b. **Major Jones criteria.** In context of prior streptococcal infection, the presence of two of five clinical features (**major Jones criteria**) is sufficient to diagnose ARF. Alternatively, the presence of one major Jones criterion plus two minor

BRIDGE TO MICROBIOLOGY

Key features of group A streptococci:

- *β hemolytic*
- *Have M protein that confers virulence*
- *Catalase negative*
- *Sensitive to bacitracin*
- *Produce streptolysins S and O*

Jones criteria (see "c" below) is also highly suggestive of the disease.

(1) **Migratory polyarthritis** involves the larger joints of the extremities, producing red, swollen, and painful manifestations. This feature is more common in adults than children.

(2) **Erythema marginatum** is a macular skin rash, often in a "bathing suit" distribution.

(3) **Sydenham's chorea** is involuntary, choreiform movements of the extremities that are seen more frequently in women.

(4) **Subcutaneous nodules** containing Aschoff bodies.

(5) **Carditis** may affect the endocardium, myocardium, or pericardium. Myocarditis causes most deaths during the acute stage. Chronic scarring of the endocardium and heart valves may lead to chronic rheumatic heart disease (RHD).

c. **Minor Jones criteria** include previous rheumatic fever, fever, arthralgias, prolonged P-R interval on ECG, elevated erythrocyte sedimentation rate, leukocytosis, and elevated C-reactive protein.

4. **Complications.** Initial episodes of ARF last weeks to months and often recur into young adulthood. Mortality is low, but the disease often leads to chronic valvulitis of the mitral and aortic valves.

B. **Rheumatic heart disease (RHD)** causes dysfunctional, deformed heart valves through chronic inflammatory insult, deposition of fibrin and platelet thrombi, and then fibrosis.

1. **Clinical features.** The patient is usually asymptomatic from puberty until young adulthood.

a. Valve leaflets become red and swollen and develop fibrinous, friable vegetations (verrucae) along lines of closure.

b. The mitral valve is most commonly (75%-80%) affected. Next in frequency is the aortic and mitral valve combination (20%-25%). The aortic valve alone is involved in 30% of patients. The tricuspid and pulmonic valves are rarely affected.

c. Valve dysfunction usually presents as a combination of stenosis and insufficiency with one predominating.

d. Fibrosis and deformity lead to "fish mouth" or "buttonhole" stenosis of the mitral valve, which may cause the patient to present with cardiac murmurs, left atrial dilatation, mural thrombi, and right ventricular hypertrophy.

e. Chronic valvulitis predisposes to infective endocarditis. CHF is the ultimate result, although it takes years to develop.

2. **Pathology**
   a. **Aschoff bodies**
      (1) Aschoff bodies are pathognomonic lesions, usually located in interstitial myocardial connective tissue, especially near vessels, but may be found elsewhere (e.g., subcutaneous nodules, pericardium).
3. **Treatment** involves prophylaxis of infective endocarditis, balloon valvuloplasty, or valve replacement.

IN A NUTSHELL

Aschoff bodies = rheumatic heart disease.

HYPERTENSIVE HEART DISEASE

Systemic hypertension, leading to left ventricular hypertrophy, produces hypertensive heart disease.

A. **Diagnosis** requires left ventricular hypertrophy; a history of hypertension; and absence of valvular, congenital, or aortic abnormalities.

NOTE

Left ventricular hypertrophy can be detected via x-ray or echocardiagram.

B. **Pathology**
   1. **Grossly,** this syndrome presents with cardiomegaly, left ventricular hypertrophy and eventual left ventricular dilatation. Hearts may weigh 2-3 times normal.
   2. **Microscopically,** hypertensive heart disease shows diffuse and scattered myocyte hypertrophy; and eventual fibrosis, atrophy, and degeneration (after years).
   3. Chronic pressure overload results in compensatory myocyte hypertrophy (myocytes cannot replicate). Myocyte hypertrophy and fibrosis decrease myocardial compliance, limit diastolic filling, increase oxygen demand, and increase the risk for myocardial ischemia.

C. **Clinical features.** Hypertensive heart disease clinically presents as CHF in one-third of cases, sudden cardiac death, stroke, or renal failure (also due to atherosclerosis and ischemia).

CONGESTIVE HEART FAILURE (CHF)

A. **Overview**
   1. CHF is often the final outcome of many cardiac diseases, resulting from the inability of the heart to provide adequate cardiac output to meet the body's metabolic demands.
   2. It is most often due to decreased myocardial contractility (e.g., myocardial infarction or fibrosis) or pressure/volume overload (e.g., hypertension).

NOTE

While CHF was previously discussed in the physiology section, it is so clinically important we thought it merited additional coverage.

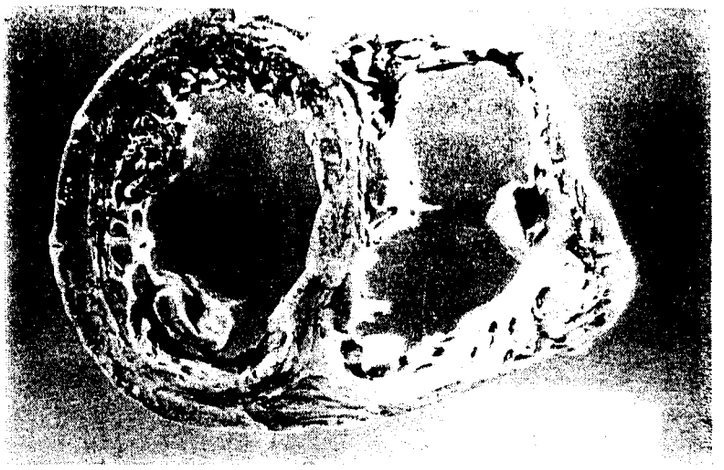


Figure 3-4. Biventricular dilatation (gross).

3. Compensatory changes that increase the workload of the heart and ultimately result in CHF:
   a. Hypertrophy and dilatation of the heart caused by compensation for increased workload or volume.
   b. Tachycardia in response to decreased stroke volume.
   c. Salt and water retention, which causes an expansion of blood volume.

B. **Left-sided heart failure** (decreased systolic ejection of blood)

1. **Etiology.** Left-sided heart failure is most often caused by ischemic heart disease and valvular disease (particularly aortic stenosis or insufficiency), hypertension, or cardiomyopathy.
2. **Clinical features**
   a. **Pulmonary congestion and edema,** resulting from pooling of blood in the pulmonary circulation, cause increased pulmonary venous pressure. Clinically, patients present with shortness of breath, orthopnea, paroxysmal nocturnal dyspnea, and cough.
   b. **Renal hypoperfusion** stimulates the renin-angiotensin-aldosterone axis, causing retention of salt and water. Fluid retention exacerbates the pulmonary edema. Hypoperfusion may also cause prerenal azotemia and acute tubular necrosis.
   c. **Cerebral hypoxia,** secondary to decreased perfusion, may lead to encephalopathy, stupor, or coma.

C. **Right-sided heart failure**

1. **Etiology.** Right-sided heart failure is most often caused by:
   a. Left-sided heart failure, causing back pressure through the lungs
   b. Cor pulmonale, valvular disease (particularly pulmonary stenosis or insufficiency), and cardiomyopathy. Mitral stenosis and insufficiency lead to back pressure on the right ventricle.
2. **Clinical features.** Right-sided failure is characterized by systemic venous congestion, leading to:
   a. Chronic passive congestion of the liver (nutmeg liver) with eventual centrilobular necrosis, followed by central hemorrhagic necrosis, and finally, (with fibrosis) cardiac sclerosis
   b. Renal hypoperfusion with salt and water retention (more severe than in left-sided heart failure)
   c. Splenomegaly
   d. Ascites, edema, pleural effusions, and cerebral hypoxia

D. **Cor pulmonale** is right ventricular failure, resulting specifically from pulmonary hypertension.

1. **Etiology**
   a. Pulmonary parenchymal disease, causing increased pulmonary vascular resistance (e.g., COPD, TB, pneumoconiosis, or carcinoid)
   b. Pulmonary vascular disease (e.g., vasculitis, shunts, or multiple emboli)
   c. Restrictive chest wall abnormalities (a rare cause)
2. **Types**
   a. **Acute cor pulmonale** is a dilatation of the right ventricles caused by massive pulmonary embolization that may cause tricuspid regurgitation.
   b. **Chronic cor pulmonale** is a gradual hypertrophy of the right ventricle, usually due to pressure overload.

CLINICAL CORRELATE

The clinical hallmarks of right-sided failure:

- *Jugular venous distension (JVD)*
- *Hepatomegaly*
- *Splenomegaly*
- *Generalized edema*

SHOCK

A. **Pathogenesis.** Shock occurs when decreased blood volume or decreased circulation leads to inadequate perfusion of body tissues and cells.

B. **Etiology**

1. **Decreased cardiac function** leads to inadequate cardiac output. Myocardial infarction, arrhythmia, tamponade, or aortic stenosis all may lead to reduced output.

2. **Reduction of blood volume,** resulting from hemorrhage, adrenal insufficiency (Addison's disease), or fluid loss (vomiting and diarrhea), may lead to poor perfusion even with adequate cardiac function.
3. **Pooling of blood in peripheral vessels** may occur as a result of the loss of vascular tone caused by bacterial toxins and vasoactive substances (free radicals, anaphylatoxins). This results in loss of vascular volume into the extracellular space.

C. **Complications. Cellular hypoxia** leads to increased anaerobic metabolism and resultant lactic acidosis, causing:
1. Encephalopathy
2. Myocardial necrosis and infarcts
3. Pulmonary edema and "shock lung" (adult respiratory distress syndrome)
4. Acute tubular necrosis in the renal cortex
5. Various hypoxic injuries to other organs

D. **Stages of compensation in shock**
1. **Compensated stage.** In this stage, early hypotension leads to reflex tachycardia and to peripheral vasoconstriction, stimulated by the CNS, causing cold, clammy, and pale extremities.
2. **Decompensated stage**
   a. Initial compensatory changes become insufficient to maintain adequate cardiac output.
   b. Decreased blood pressure, increased tachycardia, metabolic acidosis, respiratory distress, and decreased renal output may all eventually occur.
3. **Irreversible stage.** The above changes lead to irreversible cellular damage, coma, and death.

E. **Treatment.** Correction of the initial metabolic and physiologic derangements (prior to the irreversible stage) permit reversal of organ damage and prevent coma and death.

FLASHBACK TO PHYSIOLOGY

Reflex tachycardia and peripheral vasoconstriction occur via baroreceptor mechanisms. Baroreceptors are discussed in detail in the Cardiovascular Physiology chapter.

ENDOCARDITIS

A. **Infective encocarditis** is the colonization of heart valves with bacteria.
1. **Pathology**
   a. **Grossly,** the colonies form large, friable, vegetative masses, overhanging the free margins of the valve leaflet, prosthetic valve, or other cardiac defect.
   b. **Microscopically,** a mass of clot, fibrin, and bacteria is seen. In the healing phase, there is fibrosis and calcification.

c. Infective endocarditis usually involves the **mitral valve.**
d. It can involve right-sided valves in intravenous drug users, but left-sided valves may be involved as a result of paradoxic embolization or when bacteria flow through the pulmonary capillaries.

2. **Etiology**
   a. There may be implantation of colonies from transient (i.e., dental procedures, minor skin infections) or persistent bacteremia (i.e., infected intravenous catheter).
   b. **Streptococci** cause 65% of infective endocarditis, usually producing a subacute bacterial endocarditis.
   c. **Staphylococci** cause 20%-30% of infective endocarditis, usually producing acute bacterial endocarditis. Intravenous drug abuse often causes staphylococcal infection from the skin, but the spectrum of organisms is wide.
   d. **Candida** is a cause of endocarditis in intravenous drug users.
   e. **Risk factors**
      (1) Congenital cardiac anomalies with high pressure jet streams (e.g., VSD or stenoses) that produce endothelial injury.
      (2) Valvular abnormalities (e.g., mitral valve prolapse, prosthetic valves, bicuspid aortic valve, and RHD).
      (3) Immunosuppression, neutropenia, and intravenous drug abuse may contribute to infective endocarditis.
   f. **Bacterial byproducts** are swept off infected valves, resulting in the absence of an inflammatory reaction. This explains how a typically nonvirulent organism (e.g., *Streptococcus viridans*) can cause progressive disease.

3. **Types**
   a. **Acute bacterial endocarditis (ABE)**
      (1) Infection is usually caused by highly virulent organisms such as *Staphylococcus aureus* (50%) and Streptococci (35%), and it is typically seen in previously normal valves.
      (2) It often involves the tricuspid valve in intravenous drug abusers.
      (3) **Vegetations** may become large, leading to myocardial abscess formation or systemic septic emboli. Vegetations may eat through valve leaflets, producing the sudden onset of valvular incompetence and a new heart murmur (Figure 5-9).

NOTE

- *Acute bacterial endocarditis → most likely Staphylococcus aureus*
- *Subacute → more likely Streptococcus viridans*

CLINICAL CORRELATE

Endocarditis involving the right side of the heart suggests intravenous drug abuse.

b. **Subacute bacterial endocarditis (SBE)**

(1) **Etiology.** Infection is caused by organisms of low virulence, such as *Streptococcus viridans, Staphylococcus epidermidis,* Enterococci, or Gram-negative bacilli. Candida infections are rare and are usually associated with indwelling vascular catheters.

(2) **Pathology.** It is typically seen on previously abnormal valves. Regions of turbulent blood flow produced by abnormal valves permit formation of sterile platelet-fibrin aggregates on valve leaflets that become seeded during transient bacteremia. Vegetations are less bulky and less invasive than in ABE.

(3) **Clinical features.** There is an insidious onset, clinically presenting with positive blood cultures, fatigue, low-grade fever without chills, anemia, splenomegaly, and hematuria.

4. **Complications of infective endocarditis**

a. Cardiac valve perforation with acute heart failure in acute infective endocarditis

b. Myocardial abscess formation with perforation of the septum or involvement of the conduction system, leading to heart block

c. Mitral annulus and papillary muscle abscesses, leading to mitral valve prolapse

d. Right-sided septic emboli, causing pneumonia or lung abscess

e. Left-sided septic emboli, causing strokes and abscesses in the brain, spleen, and kidney

f. Nephritis may occur by:

(1) Immune complex deposition of IgM, complement component C3, and bacterial antigen in glomerular basement membranes

(2) Septic emboli, leading to renal abscess formation with rupture of the abscess into renal tubules, causing hematuria

VALVULAR HEART DISEASE

A. **Mitral valve prolapse**

1. **Etiology**

a. Prolapse of the mitral valve will lead to mitral insufficiency. The mitral leaflets (usually the posterior leaflets) billow into the left atrium during systole, leading to insufficiency.

b. Some cases may be due to a defect in connective tissue metabolism.

2. **Pathology**
   a. **Grossly,** it presents as large ballooning leaflets with elongated, drawn out, and possibly ruptured chordae tendineae.
   b. **Microscopically,** degeneration of the outer zona fibrosa and thickening of the inner zona spongiosa are seen.
3. **Incidence.** Mitral valve prolapse (MVP) is found in 7% of the United States population, most commonly in young women; it is seen in most patients with Marfan's syndrome.
4. **Clinical features**
   a. Mitral prolapse presents with a characteristic **midsystolic click** and **high-pitched murmur.** Patients are usually asymptomatic but may have dyspnea, tachycardia, chest pain, syncope, eventual CHF, or, rarely, sudden death.
   b. Prolapse may coincide with tricuspid or pulmonary valve disease. It may also be associated with psychiatric conditions (e.g., anxiety, depression) through an unknown mechanism.
5. **Complications** include atrial thrombosis, calcification, infective endocarditis, embolization (to brain), rupture of chordae, arrhythmias, and sudden death. MVP can also lead to mitral regurgitation/insufficiency and premature ventricular contractions (PVCs).

B. **Mitral stenosis**

1. **Pathogenesis.** Mitral stenosis is due to scarring, calcification, and fusion of the mitral valve, interfering with its opening (Figures 3-5 and 3-6). It is most commonly caused by rheumatic heart disease.

Figure 3-5. Normal mitral valve (gross).

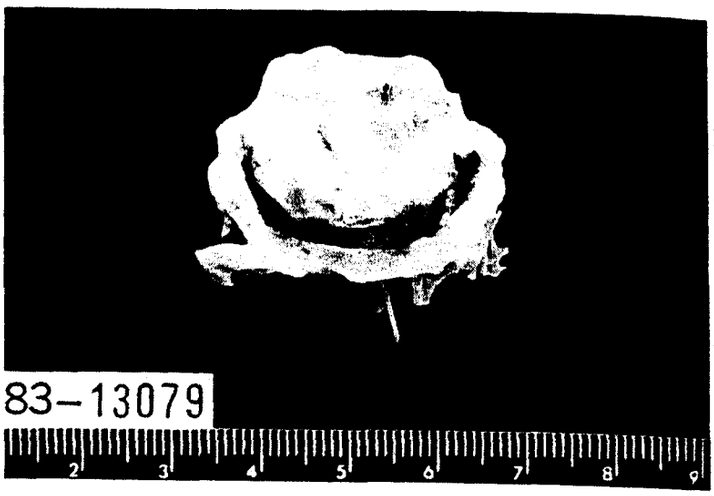


Figure 3-6. Mitral valve stenosis: "fish mouth" (gross).

2. **Clinical features**
   a. Mitral stenosis presents with increased left atrial pressure and an enlarged left atrium. Stenosis may be combined with mitral valve prolapse.
   b. An **early diastolic opening snap** is characteristic of mitral stenosis. Severe mitral stenosis can lead to backward failure (e.g., CHF) if the valve fails to open sufficiently.
3. **Complications.** Prolonged stenosis, producing left atrial enlargement, may eventually produce chronic atrial fibrillation, which predisposes to atrial thrombosis.

C. **Aortic valve insufficiency**

1. **Acute**
   a. It may lead to left ventricular failure due to increased left ventricular filling pressure, inadequate stroke volume, decreased diastolic filling time (due to reflex tachycardia), and myocardial ischemia.
   b. Acute insufficiency may result from perforations or tears from infective endocarditis.
2. **Chronic**
   a. The left ventricle will dilate and hypertrophy to accommodate the gradual increase in regurgitating diastolic volume and to maintain adequate net cardiac output.
   b. A wide pulse pressure (clinically seen as bounding pulses) causes reflex tachycardia.
   c. It may be due to a congenitally bicuspid aortic valve, RHD, or syphilis.

NOTE

Insufficiency = regurgitation

Backflow through the aortic valve leads to ↑ LV volume, therefore ↑ filling pressure leading to LV failure.

D. **Aortic valve stenosis**

1. **Etiology**
   a. Calcific (degenerative) heart disease causes 90% of stenotic valves.
   b. The aortic valve is affected somewhat less than the mitral valve in RHD.
   c. Congenital heart disease causes a higher percentage of stenosis since the advent of penicillin.
2. **Incidence** increases with age.
3. **Pathology**
   a. It is often associated with a congenitally abnormal (e.g., bicuspid) aortic valve.
   b. **Grossly,** it presents as large calcified masses with thickening and fibrosis of valve cusps without fusion of valve commissures; rheumatic aortic valve stenosis involves fusion of the valve leaflets.
4. **Clinical features**
   a. It presents with angina, syncope, and CHF; it is often asymptomatic until late in the course of the disease.
   b. Signs of increasing stenosis include decreasing peripheral pulse pressure, slowing of the carotid upstroke, and increasing left ventricular hypertrophy as a result of chronic pressure overload.
   c. A systolic ejection click is characteristic of aortic valve stenosis.
5. **Complications.** It may lead to sudden death, secondary to an arrhythmia or CHF.
6. **Treatment.** Definitive treatment is surgical replacement of the aortic valve.

E. **Mitral annulus calcification** is a deposition of calcium within the ring of tissue surrounding the base of the mitral valve. It is a degenerative, noninflammatory disorder.

1. It may lead to mitral regurgitation because of the inability of the mitral valve ring to close during contraction of the left ventricle.
2. It usually occurs in the elderly and is associated with IHD.

F. **Valve replacement**

1. Both mechanical and bioprosthetic (usually porcine) valves may be indicated in:
   a. Mitral and aortic valve stenosis
   b. Mixed mitral valve stenosis and insufficiency
   c. Mitral and aortic valve insufficiency (regurgitation)

2. **Complications** that occur in 10% of patients a year, include:
   a. Thromboembolism
   b. Infective endocarditis
   c. Paravalvular leak through the suture line (a problem exacerbated by calcification of the mitral annulus)
   d. Microangiopathic hemolysis (mechanical trauma to passing RBCs as a result of the presence of an artificial valve). This is diagnosed by observing fragmented RBCs (schistocytes) on the blood smear.

MYOCARDITIS

A. **Overview.** Myocarditis is an inflammation of the myocardium that may lead to necrosis. **Noninfectious myocarditis** may be due to a hypersensitivity reaction seen in collagen vascular diseases, rheumatic fever, SLE, and drug allergies. It may also contribute to pathology in viral myocarditis. Trauma, which produces inflammation or necrosis, may also produce myocarditis.

B. **Pathology**

1. **Grossly,** it presents with dilatation and hypertrophy of all four chambers. A diffuse but patchy hemorrhage in the myocardium with eventual small, pale foci of fibrosis can be seen.
2. **Microscopically,** myocarditis is characterized by focal inflammatory lesions with:
   a. A neutrophilic infiltrate, abscess, or granuloma in bacterial myocarditis
   b. A mononuclear infiltrate and necrosis in viral myocarditis
   c. An eosinophilic infiltrate with giant cell and granuloma formation in Fiedler's myocarditis

C. **Etiology**

1. **Viral myocarditis** is the most common form of myocarditis (especially **coxsackie B virus**).
   a. Polio, rubella, and influenza viruses have also been described as etiologic agents.
   b. It is usually self-limited, but it may be recurrent and lead to cardiomyopathy and death.
   c. Approximately one-third of AIDS patients show focal myocarditis on autopsy.
2. **Bacterial myocarditis** may be due to diphtheria, meningococci, or other bacteria. These bacteria may damage the heart directly or via secreted toxins, such as diphtheria toxin.

BRIDGE TO MICROBIOLOGY

Coxsackie B viruses are positive-sense RNA viruses belonging to the Picornavirus family.

3. **Protozoal etiology**
   a. *Trypanosoma cruzi* causes **Chagas' disease,** which is characterized by trypanosome-containing myocardial pseudocysts, causing myocardial necrosis. Fifty percent of the population is infected in endemic areas of South America and it is an important cause of congestive heart failure in that area of the world.
   b. Toxoplasmosis also causes myocardial pseudocysts.

D. **Clinical features**

1. Myocardial involvement appears days to weeks after the primary infection. There is a variable severity, depending on the etiology.
2. Myocarditis may be asymptomatic with EKG abnormalities only, or it may present with the acute onset of dyspnea, tachycardia, weakness, or severe CHF. Edema is common to all forms of myocarditis.
3. It may have a protracted or a fulminant course; most patients recover fully without long-term adverse effects.

CLINICAL CORRELATE

Romaña's sign—unilateral swelling of the eyelid—is a sign of Chagas' disease.

PERICARDIAL EFFUSION

Pericardial effusion is leakage of fluid (transudate or exudate) into the pericardial space. It may result in cardiac tamponade, in which the collection of fluid compresses the heart, limiting filling during diastole, and decreasing cardiac filling.

A. **Serous effusion** results from hypoproteinemia or CHF. It usually develops slowly, rarely causing cardiac compromise.

B. **Serosanguineous effusion** is usually due to trauma (e.g., cardiopulmonary resuscitation), tumor, or TB. It rarely causes cardiac compromise.

C. **Hemopericardium** occurs when blood flows into the pericardial sac as a result of trauma, ventricular rupture (after myocardial infarction), or aortic rupture. There is no inflammatory infiltrate. This condition can quickly cause cardiac tamponade and death.

CLINICAL CORRELATE

Hemopericardium can be a complication of vigorous CPR.

CONGENITAL ABNORMALITIES OF VESSELS

A. **Berry aneurysms** are focal weakenings in **cerebral vessel walls,** resulting in an outpouching. They are most common at branch points in the anterior circle of Willis and at the bifurcation of the middle cerebral artery. Symptoms are rare before age 20, after which time they may burst and cause a subarachnoid hemorrhage.

B. **Arteriovenous (AV) fistula** is a rare abnormal communication between a vein and an artery.

1. By diverting blood from the arterial to the venous circulation, it increases venous return, increases the workload to the right heart, and may, therefore, cause right heart failure.
2. AV fistulas may also form as a result of trauma.

ARTERIAL HYPERTENSION

A. **Clinical features**

1. Arterial hypertension is defined as a consistent diastolic pressure over 100 mmHg (if the patient is over 60 years of age) or over 90 mmHg (if the patient is under 60 years of age with a systolic pressure over 160 mmHg.).
2. Hypertension causes hypertensive heart disease with progressive thickening of the left ventricle, myocyte dropout, fibrosis, and eventual heart failure.

B. **Morbidity and mortality**

1. Hypertension is the second leading cause of cardiac mortality after ischemic heart disease.
2. Hypertension is strongly associated with both stroke and myocardial infarction. It may also lead to CHF, renal failure, coronary and peripheral artery disease, and aortic dissection.
3. Mortality has been declining as a result of early recognition, antihypertensive therapy, and control of obesity.

C. **Essential (primary) hypertension** is idiopathic and accounts for approximately 90% of cases. The pathophysiology is unknown, but it may be due to genetic or environmental factors, most likely resulting in increased systemic vascular resistance or increased cardiac output. Type A personality, obesity, stress, high-salt diet, and oral contraceptives increase the risk; it is most common in African-American males around 40 years of age.

D. **Secondary hypertension** is hypertension resulting from other diseases, most commonly renal disease.

ATHEROSCLEROSIS

Atherosclerosis involves the progressive formation of elevated **fatty plaques (atheromata)** in the intima of large- and medium-sized muscular and elastic arteries. The atheromata cause narrowing of the vessel lumen, weakening of the media, and possibly progression to ulceration, calcification, thrombosis, intralesional hemorrhage, or

aneurysm formation. This disorder affects primarily the coronary, cerebral, and iliac arteries and the aorta. It accounts for 50% of all deaths in the United States. Death occurs mainly from myocardial or cerebral infarcts.

A. Pathology

1. **Grossly,** atherosclerosis presents with:
   a. White or pale yellow plaques 0.5-1.5 cm in diameter bulging into the lumen with a soft "gruel-like" center
   b. Lesions occur (in order of frequency) in the abdominal aorta, coronary arteries, popliteal arteries, descending thoracic aorta, internal carotid arteries, and circle of Willis
2. **Microscopically,** atherosclerosis presents (from inside the lumen to the outer vessel wall) as:
   a. A fibrous cap composed of smooth muscle cells, collagen, connective tissue matrix, and scattered leukocytes
   b. A cellular zone composed of smooth muscle cells, macrophages, and lymphocytes
   c. A central core composed of necrotic cells, cholesterol clefts, lipid-filled foam cells, and plasma proteins
   d. Proliferating capillaries when lesions are well-advanced
3. **Complicated plaques** are seen in advanced disease. They arise when calcification and thickening cause ischemia of the intima. Fissure, ulceration, and rupture of atheromas into the lumen may cause:
   a. Thrombus formation with occlusion of the vessel, leading to infarction of the tissue it supplies
   b. Cholesterol emboli
   c. Hemorrhage into the lesion
   d. Aneurysmal dilatation
4. **Fatty streaks** have the following characteristics.
   a. They are elevated, poorly demarcated, yellow intimal lesions less than 2 mm wide and 1 cm long.
   b. They may be present in children as young as 1 year old and may or may not evolve into atheromas.
   c. They are composed of lipid-containing cells (macrophages and smooth muscle cells), collagen, elastic fibers, proteoglycans, and extracellular lipid.
   d. They are most common in the thoracic aorta (< 1 year) and coronary arteries.

NOTE

Foam cells = macrophages after lipid ingestion.

B. **Etiology of atheromatous plaques**

1. **Response to injury**
   a. Endothelial injury may be due to hypertension, hyperlipidemia, chemicals in tobacco smoke, diabetic angiopathy, and gross physical or chemical injury.
   b. Injury may lead to increased permeability of plasma proteins, platelet and inflammatory cell adherence, and thrombus formation at the site.
   c. Chemical mediators from the above cells may induce migration and proliferation of smooth muscle cells from the media into the intima.
   d. Production of abundant connective tissue matrix (collagen, elastic fibers, proteoglycans) by smooth muscle cells follows with ingrowth of intimal capillaries from the vasa vasorum for nourishment. This may lead to subsequent leakage of more plasma proteins, finally resulting in the deposition and accumulation of lipid in the plaque.
2. **The loss of growth control hypothesis** suggests that smooth muscle proliferation in the media may be the initial event.

C. **Risk factors**

1. **Hypertension.** The risk of atherosclerosis correlates more closely with diastolic than with systolic pressure.
2. **Cigarette smoking.** The death rate from ischemic heart disease is 70%-200% higher in men who smoke at least one pack per day than in nonsmoking men.
3. **Hyperlipidemia**
   a. Elevated serum cholesterol levels, especially low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL)
   b. Hyperlipidemia may be due to genetic (e.g., familial hypercholesterolemias), dietary (e.g., high cholesterol and saturated fat intake), other clinical conditions (e.g., nephrotic syndrome, hypothyroidism), or a sedentary lifestyle.
   c. Elevated high-density lipoproteins (HDLs) may decrease risk because HDL transports cholesterol out of tissues back to the liver, while LDL transports cholesterol from the liver to the tissues.
4. **Diabetes** causes damage to arterioles by depositing hyaline material in their walls and reducing blood flow.
5. **Increasing age.** Significant atherosclerosis is rarely seen in patients younger than 30 years of age; it becomes symptomatic in patients in their fifties and sixties.
6. Incidence is higher in men, in postmenopausal women, and in individuals with a positive family history.

Figure 3-7. Coronary artery atherosclerotic occlusion, old (microscopic).

7. Sedentary lifestyle, obesity, oral contraceptives, stress, and a compulsive, workaholic behavior pattern (type A personality) all increase the risk.

D. **Clinical features**

1. Atherosclerosis may be asymptomatic for decades.
2. Ischemia due to gradual vessel occlusion (e.g., gangrene of the lower extremities, intermittent claudication) may eventually develop.
3. Infarction due to sudden occlusion by thrombosis or embolization (e.g., myocardial infarction, renal artery occlusion) is the most dramatic sign. Twenty-five percent of cases of coronary atherosclerosis present with sudden death.
4. Aneurysm formation with subsequent rupture (e.g., abdominal aortic aneurysm) may also be a presenting sign.

ARTERIOLOSCLEROSIS

Arteriolosclerosis is a diffuse thickening of arterioles and small arteries, resulting in narrowing of the lumen and ischemia of involved tissue.

A. **Hyperplastic arteriolosclerosis** is associated with malignant hypertension or necrotizing vasculitis and is characterized by "onion-skin hyperplasia," i.e., concentric thickening of the intima, deposition of basophilic ground substance, smooth muscle proliferation, and hypertrophy of the adventitia.

B. **Hyaline arteriolosclerosis** is associated with diabetes, hypertension, and old age. It is characterized by hyaline thickening of

NOTE

The kidney is particularly vulnerable to arteriolosclerosis.

arterioles that narrows the vessel lumen. This form of arteriolosclerosis is further characterized by eosinophilic material (thickened basement membrane of endothelial and smooth muscle cells) in the intima and media and is a degenerative process. It is best recognized in the arterioles of adipose tissue, where the vessel walls appear as thick as the diameter of the lumen.

EMBOLISMS

Embolisms may arise from solid, liquid, or gaseous masses transported within the vessels and originating from thrombi (98%), fat (bone fractures), atheromas, gases (deep sea divers), or amniotic fluid (pregnancy).

A. **Emboli arising from the venous circulation** involve the pulmonary circulation (pulmonary emboli). These may paradoxically involve the systemic circulation via a right-to-left cardiac shunt (e.g., atrial septal defect), which was not previously known to exist.

B. **Emboli arising from the arterial circulation** involve nonpulmonary structures.

1. Seventy-five percent arise from cardiac mural thrombi due to myocardial infarction. **Grossly,** mural thrombi appear grey-red with alternating light and dark lines (lines of Zahn), which represent clotted plasma and red blood cells (RBCs), respectively.
2. Arterial emboli most commonly involve the legs, then the brain, other viscera, and the arms.

ANEURYSMS

Aneurysms are focal, abnormal, dilatations of arterial vessels as a result of wall weakness. They may lead to rupture, which is a recognized cause of sudden death, compression of nearby structures, and thrombus formation and embolism, which may cause infarction of distal organs or structures.

A. **Atherosclerotic aneurysms** are secondary to atheroma formation. They usually occur in the abdominal aorta below the renal arteries, are associated with hypertension, and are found in men over 50 years of age. Fifty percent of atherosclerotic aneurysms over 6 cm in diameter will rupture within 10 years.

B. **Syphilitic aneurysms** are due to chronic damage to the vasa vasorum of the aortic media by syphilitic aortitis. This damage results in obliterative endarteritis, ischemia, and smooth muscle cell atrophy. It usually occurs in the ascending aorta and may impinge on the aortic valve, causing aortic insufficiency due to dilatation of the valve ring.

C. **Microaneurysms** may appear in cerebral vessels as a result of hypertension and in retinal vessels as a result of diabetic vasculitis.

D. **Dissecting aneurysms** are due to degeneration of the tunica media, which allows blood from the lumen to enter an intimal tear and dissect through the layers of the media. They most frequently occur in the **aorta.**

1. They may progressively spread into aortic branches (e.g., renal or coronary arteries), leading to compression and obstruction of the lumen of the branch.
2. Etiology is unknown, but hypertension and Marfan's disease are predisposing factors.

E. **Berry aneurysms** were previously discussed.

BRIDGE TO BIOCHEMISTRY

In Marfan's disease, there is a defect in the gene for fibrillin on chromosome 15q. Fibrillin is a 350-kD molecule, a glycoprotein present in connective tissue, particularly the suspensory ligament of the lens, the walls of blood vessels, and the skin.

VASCULITIS

Vasculitis is an inflammation of the vessels that may be localized (due to trauma, infections, toxins) or systemic. Multifocal vasculitis may lead to widespread, patchy necrosis and thrombi formation and is usually due to an immune reaction.

A. **Polyarteritis nodosa (PAN)** is a systemic necrotizing vasculitis of small- and medium-sized muscular arteries (often at bifurcations).

1. **Etiology**
   a. Hepatitis B antigenemia can be demonstrated in 30% of cases.
   b. Essentially all cases of PAN are thought to be due to **antigen-antibody complexes.**
   c. Autoantibodies may also play a role when they form complexes with self-antigens. P-ANCA (perinuclear antineutrophil cytoplasmic autoantibodies) are frequently observed in PAN and may correlate with disease activity.
2. **Pathology**
   a. **Grossly,** PAN presents as up to 1-cm segmental aneurysmal dilatations in vessels. It is seen predominantly in the kidneys, heart, and gastrointestinal tract; the pulmonary circulation is spared.
3. **Clinical features**
   a. Symptoms depend on the system involved. Patients most commonly have low-grade fever, weakness, and weight loss. They may also have abdominal pain, hematuria, renal failure, hypertension, and leukocytosis.
   b. PAN is most common in young adults.

BRIDGE TO IMMUNOLOGY

Antigen-antibody (Ag-Ab) complexes precipitate onto vessel walls, where they fix complement and attract neutrophils by means of gradients of C5a fragments. Neutrophils phagocytose the complexes and discharge lysosomal granules that destroy smooth muscle and elastic fibers.

IN A NUTSHELL

Headache + tenderness at temples + elevated ESR = temporal arteritis.

B. **Temporal (giant-cell) arteritis** is a granulomatous inflammation of small- and medium-sized arteries, **particularly extracranial arteries** (especially the temporal artery). This is perhaps the most common form of vasculitis. **Etiology** is unknown.

1. **Clinical features**
   a. Giant cell arteritis occurs in both males and females, usually greater than 50 years old, and affects mainly the cranial and most commonly the temporal arteries.
   b. It clinically presents with:
      (1) **Headache and facial pain** (the most common symptoms)
      (2) Fever, malaise, weight loss, muscle aches, anemia, claudication of the jaw, visual disturbances in 40% of cases, and tender, firm temporal arteries
      (3) **Elevated ESR,** as in all inflammatory diseases
      (4) Blindness, if not treated early (due to occlusion of ophthalmic artery)

D. **Hypersensitivity (leukocytoclastic) angiitis** affects small vessels (i.e., arterioles, venules, capillaries) predominantly in the skin. It may also affect vessels, lungs, kidneys, and other organs simultaneously and may cause crescentic glomerulonephritis. Hypersensitivity angiitis may be distinguished from PAN by the involvement of smaller vessels. Lesions are usually all in the same stage at the same time.

1. **Etiology.** Immune complexes are thought to be involved because it is often precipitated by a specific antigen, such as bacteria (e.g., Streptococcus), drugs (penicillin), tumor antigens, or serum sickness. The disease remits if the offending agent is removed.

IN A NUTSHELL

If you see gangrene in a young smoker → think Buerger's disease.

E. **Thromboangiitis obliterans (Buerger's disease)** is a recurrent acute and chronic inflammatory disorder of small- and medium-sized arteries and veins, causing **segmental thrombosis** that occurs in the extremities and may also affect adjacent nerves. It occurs **almost exclusively in cigarette smokers** less than 35 years of age.

1. **Etiology.** Possible causes include a genetic predisposition, an immunologic reaction, and a direct toxic response (tobacco).

F. **Wegener's granulomatosis** consists of a triad of necrotizing vasculitis of lungs and airways, necrotizing granulomas of the upper respiratory tract, and necrotizing glomerulitis. It occurs in men more often than women. Patients are usually older than 50 years of age. **Etiology** is unknown.

G. **Takayasu's arteritis (pulseless disease)** is a granulomatous inflammation of medium-to-large arteries, often branches of the aortic arch.

1. **Etiology**. The cause is unknown.

H. **Kawasaki's disease (mucocutaneous lymph node syndrome)** was first described in Japan and is still more common there.

1. **Epidemiology.** The disease is usually seen in young children, but adult patients have been described (rare).
2. **Etiology.** The cause is unknown. An RNA-dependent DNA polymerase has been found in some lesions, suggesting a viral etiology.
3. **Pathology. Microscopically,** it presents with inflammation and necrosis of the entire vessel wall and possible aneurysm formation.
4. **Clinical features.** Kawasaki's disease is an acute syndrome consisting of:
   a. Fever, conjunctivitis, erythema and erosions of the oral mucosa, a generalized maculopapular skin rash, and adenopathy
   b. A mortality rate of 1%–2% as a result of coronary vasculitis or coronary aneurysm, thrombosis, or rupture
   c. Self-limited course

VENOUS DISEASE

A. **Thrombophlebitis** is inflammation and thrombus formation of the veins. Ninety percent of cases occur in the **deep veins of the leg** (i.e., deep venous thrombosis).

1. **Pathology.** Factors involved in thrombus formation (Virchow's triad) are endothelial injury, alterations in blood flow, and hypercoaguability of blood. Thrombi grossly appear blue-red.
2. **Clinical features**
   a. Thrombophlebitis may be associated with or may be secondary to:
      (1) Clotting disorders (deficiency of antithrombin III, protein C, or protein S). In these deficiencies, normal clot dissolution (fibrinolysis) is abnormally slow.
      (2) Heart disease (CHF, myocardial infarction, valvular disease), leading to sluggish flow
      (3) Immobilization (including bed rest), slowing venous flow
      (4) Neoplasia, sometimes producing enzymes that promote clotting

(5) Advanced age with sclerotic veins and slow flow
(6) Pregnancy with obstruction of pelvic veins
(7) Oral contraceptives, which activate some clotting factors
(8) Tissue injury (postoperative course, trauma), which also activates clotting, sometimes systemically

b. Thrombi may cause:
(1) Embolization, particularly to the lungs
(2) Bacterial superinfection, producing a septic nidus
(3) Postphlebitic syndrome (predisposition of recurrent thrombosis due to loss of venous valves)
(4) Recanalization of the thrombus, restoring more normal flow

c. Clinically, thrombophlebitis presents insidiously with few symptoms (localized pain, erythema, and edema). It often presents initially as a **pulmonary embolism** or as multiple emboli; a large embolus may cause sudden death.

B. **Venous occlusion** may occur as a result of thrombophlebitis, deep venous thrombosis, or obstruction of outflow (pregnancy).

C. **Varicose veins** are dilated, tortuous veins, most likely resulting from increased intraluminal pressure and inadequate external support. They occur most frequently in the superficial veins of the lower extremities. They are more common in women.

1. **Pathogenesis.** Varicose veins are associated with the venous stasis of pregnancy, obesity, compression by tumors, prolonged immobility of legs, and congenital defects in venous walls (including valves). They may result in venous thrombosis and valve damage.

2. **Clinical features.** Varicosites present with edema, thrombosis, stasis dermatitis, and ulcerations. Unlike venous abnormalities of the deep veins of the lower extremities, varicose veins are rarely a source of emboli.

NOTE

Some of the vasculitides affect the veins and venules—see the previous section on "Vasculitis."

VASCULAR NEOPLASMS

Vascular neoplasms include all of the neoplastic growths of the vascular endothelial cells, forming well-defined endothelial-lined vascular channels in benign tumors, or ill-defined masses of anaplastic endothelial cells in malignant tumors.

A. **Benign tumors**

1. **Hemangiomas**

a. **Capillary hemangiomas** form unencapsulated well-defined masses of capillaries with a small amount of connective tissue that usually occur in the skin and mucous membranes.

b. **Cavernous hemangiomas** form sharply defined, sponge-like tumors composed of large, dilated, cavernous vascular spaces. They usually occur on the skin, mucous membranes, and viscera and are rarely clinically significant except for their cosmetic effects.

c. **von Hippel-Lindau disease** is a syndrome of multiple cavernous hemangiomas involving the cerebellum, brain stem, liver, pancreas, and eyes. It is associated with renal cysts and renal cell carcinoma. This disease is transmitted via an autosomal dominant pattern with the gene localized to chromosome 3p.

2. **Vascular ectasias** (telangiectasias) are actually a developmental abnormality but can closely mimic benign vascular neoplasms. They may be composed of abnormal aggregations of arterioles, capillaries, or venules.

a. **Nevus flammeus** is a flat birthmark on the head or neck that usually spontaneously regresses.

b. **Port wine stain** may grow proportionately with the child and may be associated with **Sturge-Weber syndrome,** a nevus formation in the skin supplied by the trigeminal nerve, and associated with glaucoma, meningeal angiomas, and mental retardation.

c. **Spider telangiectasias** are a radial array of tiny arterioles, commonly occuring in **pregnant women** and patients with **hepatic cirrhosis.** In men, they may be related to elevated estrogen levels occurring as a result of liver disease (e.g, alcoholism).

B. **Malignant tumors**

1. **Hemangiosarcomas** are growths of atypical, anaplastic endothelial cells that usually metastasize and are associated with a high mortality.

a. **Gross pathology.** Hemangiosarcomas most commonly occur in skin, breast, liver, and soft tissues. They are usually sharply defined red nodules, which become large, pale, soft masses.

b. **Microscopic pathology.** Hemangiosarcomas show varying degrees of anaplasia and vessels of different sizes and shapes. Vessels are often merely slit-like spaces.

2. **Hepatic angiosarcomas** are tumors caused by toxic exposures.

3. **Kaposi's sarcoma** was once a rare, slowly progressive disease seen in older men of Mediterranean or African descent or immunosuppressed transplant patients. It is now seen in one-third of **AIDS patients,** most frequently in homosexual males.

This form of the disease may be more aggressive and frequently disseminates.

a. **Pathology**

(1) **Grossly,** it presents as multiple **violaceous nodules** that may remain confined to the skin or may disseminate.

(2) **Microscopically,** it presents as a proliferation of endothelial cells, spindle cells, and inflammatory cells with RBCs scattered throughout slit-like vascular spaces.

b. **Prognosis.** Kaposi's sarcoma rarely causes death. It is responsive to chemotherapy and interferon-α (INF-α), but it usually spreads relentlessly in AIDS patients.

Endocrine Pathology

Endocrine pathology is primarily concerned with the hypothalamic-pituitary-end organ axis. Knowledge of the complex homeostatic feedback mechanisms affecting the hypothalamus and pituitary is critical to making an accurate diagnosis of hyper- or hypofunctioning of the endocrine glands or organs. In general, hyperplasia of glands implies an excess of stimulating hormone, while adenomas and carcinomas may arise completely independently of normal regulatory hormone secretion. Hyperplasias are almost always functional. In contrast, adenomas vary in the amount of functional product they secrete; moreover, their responses to regulatory hormone vary considerably. Carcinomas are usually the least functional and are usually independent of regulatory hormonal influence.

HYPOTHALAMUS AND PITUITARY GLAND

A. **Lesions of the hypothalamus**

1. **Destructive lesions** include tumors such as craniopharyngiomas, gliomas, hamartomas, and inflammatory conditions (e.g., sarcoidosis).
2. **Craniopharyngiomas** arise from ectodermal remnants of Rathke's pouch, forming the most common pituitary tumor in children. Pathology shows stratified squamous epithelium with areas of keratinization and cysts. Lamellar bone deposits and calcium may be seen. Malignant transformation is rare. The tumor may be detected on x-ray by its opaque calcifications.

FLASHBACK TO PHYSIOLOGY

The hypothalamus produces growth hormone-releasing hormone (GHRH), somatostatin, dopamine, gonadotropin-releasing hormone (GnRH), corticotropin-releasing hormone (CRH), antidiuretic hormone (ADH), thyrotropin-releasing hormone (TRH), and oxytocin.

B. **Anterior pituitary hyperfunction**

1. **Etiology.** Most cases of anterior pituitary hyperfunction are caused by **adenomas,** which usually secrete prolactin, growth hormone, or adrenocorticotropic hormone (ACTH).
2. **Clinical syndromes** correspond to the hormone secreted.
   a. **Hyperprolactinemia** (amenorrhea-galactorrhea syndrome) results from elevated serum prolactin associated with pituitary adenomas (prolactinoma). It is the **most common pituitary tumor**. In women, it results in **amenorrhea and galactorrhea**; in men, this tumor may result in galactorrhea and infertility.
   b. **Excess growth hormone**
      (1) **Gigantism** occurs if there is excessive GH secretion **before the growth plates are fused** (i.e., before the end of puberty). Excessive skeletal growth may result in heights up to 9 feet tall.
      (2) **Acromegaly** occurs if there is excessive secretion **after closure of the epiphyseal plates**. There is a gradual coarsening of facial features (i.e., thick lips, protruding jaw, large tongue) and enlargement of the hands and feet. It may be associated with diabetes mellitus, hypertension, osteoporosis, and other symptoms associated with space-occupying lesions in the pituitary region, such as visual field defects.
   c. **Cushing's disease** is caused by ACTH-secreting tumors. Lesions are usually small and rarely cause mass effect. Cushing's disease is discussed later this section.

C. **Anterior pituitary hypofunction** is usually manifested as panhypopituitarism, resulting from the destruction of at least 75% of the adenohypophysis.

1. **Clinical features** include symptoms of hypothyroidism, hypoadrenalism, and hypogonadism. Growth hormone deficiency in children results in **primary dwarfism** with normal limb and skull proportions.

D. **Posterior pituitary hypo- and hyperfunction**

1. **Diabetes insipidus (DI)** is due to insufficient or absent antidiuretic hormone (ADH).
   a. **Etiology.** Disorders involving the hypothalamus or neurohypophysis (e.g., malignancy, meningitis, TB, sarcoid, postsurgical trauma to base of skull) may all cause central diabetes insipidus. **Nephrogenic diabetes insipidus** is caused by a lack of renal response to ADH.

CLINICAL CORRELATE

A pituitary tumor may impinge on the optic chiasm, producing a bitemporal hemianopsia (loss of peripheral visual fields).

IN A NUTSHELL

Diabetes insipidus (DI):

- *Polydipsia*
- *Polyuria*
- *Large volumes of dilute (hypotonic) urine*
- *High serum osmolality*
- *Hypernatremia*

Central DI responds to exogenous ADH therapy; nephrogenic DI does not because renal receptors do not respond to ADH.

b. **Clinical features** include **polydipsia** and **polyuria** with excretion of **large volumes of dilute urine**, even during states of dehydration.

ADRENAL GLANDS

A. Adrenal cortical hyperfunction

1. **Cushing's syndrome** is caused by cortisol excess.
   a. **Etiology.** Cushing's syndrome may take one of four distinct forms, depending on its cause.
      (1) **Pituitary Cushing's syndrome** (approximately two-thirds of the cases of Cushing's). Pituitary or hypothalamic dysfunction is the most common noniatrogenic cause. It is caused by basophilic adenomas, referred to as **Cushing's disease,** or more commonly, by multiple corticotroph microadenomas. Pituitary Cushing's syndrome is characterized by **bilateral adrenal hyperplasia** and **elevated serum ACTH.**
      (2) **Adrenal Cushing's syndrome** is usually caused by an **adrenal adenoma.** It is characterized by **low serum ACTH**.
      (3) **Ectopic Cushing's syndrome** is caused by ectopic secretion of ACTH, most commonly by **bronchogenic cancer**.
      (4) **Iatrogenic Cushing's syndrome** is rather common and is caused by exogenous administration of glucocorticoids or ACTH.
   b. **Clinical features** usually result from excess cortisol but may also be due to excess aldosterone, corticosterone, or adrenal androgens. The syndrome is most common in women in the 20-40-year-old age group. Patients exhibit **hypertension, abnormal glucose tolerance** (frank diabetes 20%), **truncal obesity, muscle wasting** in the extremities, **moon facies, buffalo hump**, cutaneous **striae, osteoporosis,** hirsutism and amenorrhea in women, weight gain, edema, weakness, fatigue, susceptibility to infection and personality disturbances. Children show growth retardation, delayed skeletal maturation, and precocious puberty if associated with adrenal androgens.
2. **Primary hyperaldosteronism (Conn's syndrome)** is due to increased aldosterone secretion, producing **sodium retention, increased total plasma volume,** increased renal artery pressure, and **inhibition of renin secretion**.
   a. **Etiology.** An adrenal adenoma secreting aldosterone is the most common cause.

b. **Clinical features** include sodium retention, extracellular fluid expansion, and potassium depletion with diastolic hypertension, weakness, fatigue, polyuria, polydipsia, and headache.

c. **Laboratory values** reveal **hypokalemia, low renin levels, metabolic alkalosis, hypernatremia**, and (for adenomas) failure to suppress aldosterone with salt loading.

3. **Secondary hyperaldosteronism**

a. **Etiology.** The causes are decreased renal blood flow or perfusion pressure, edematous states with sodium retention, renin-producing neoplasms, and **Bartter's syndrome,** which is characterized by juxtaglomerular cell hyperplasia, hyperreninemia, hyperaldosteronism, and failure to thrive; it is often associated with low blood pressure.

b. **Laboratory values** include **high renin levels**, hypernatremia, and hypokalemia. Secretion of aldosterone is triggered by elevated renin-angiotensin levels.

B. **Adrenal cortical hypofunction**

1. **Acute adrenocortical insufficiency** can be caused by:

a. **Rapid withdrawal of exogenous steroids** in patients with chronic adrenal suppression

b. **Stress** (e.g., trauma, surgery, infection), Addison's disease, or chronic adrenal suppression caused by administration of exogenous corticosteroids

c. **Adrenal apoplexy,** such as in the **Waterhouse-Friderichsen syndrome**: a massive, sudden adrenal hemorrhage usually associated with meningococcal septicemia

2. **Chronic or primary adrenocortical insufficiency (Addison's disease)**

a. **Etiology.** Tuberculosis was once the most common cause. The most common etiology today is idiopathic (probably autoimmune).

b. **Pathogenesis.** To produce clinical insufficiency, 90% of the adrenal gland must be nonfunctional.

c. **Clinical features** are due to insufficient cortisol and aldosterone secretion, leading to weakness, weight loss, anorexia, nausea, vomiting, hypotension, skin pigmentation, hypoglycemia with prolonged fasting, inability to tolerate stress, and abdominal pain.

d. **Laboratory values** show decreased serum sodium and chloride with metabolic acidosis and increased serum potassium. **ACTH levels are high**.

NOTE

Pigmentation of the skin in Addison's is due to ACTH also having a weak stimulatory effect on melanocytes. ACTH and melanocyte-stimulating hormone (MSH) share amino acid sequences.

3. **Secondary adrenocortical insufficiency**
   a. **Etiology.** Causes include metastases, irradiation, infection, and infarction, affecting the hypophysial-pituitary axis and resulting in **decreased ACTH**.
   b. **Clinical features.** Secondary insufficiency usually produces less mineralocorticoid malfunction and less pigmentation.

C. **Adrenal neoplasms**

1. **Adrenal adenomas**
   a. **Clinical features.** Adrenal adenomas are mostly asymptomatic and nonsteroid-producing.
2. **Adrenal carcinomas**
   a. **Clinical features.** Adrenal carcinoma is relatively rare and usually very malignant. Greater than 90% are steroid-producing (often more than one steroid).
3. **Pheochromocytoma**
   a. **Etiology.** Pheochromocytoma is a neoplasm of neural crest-derived chromaffin cells that **secrete catecholamines,** resulting in **hypertension**.
   b. **Clinical features** are related to catecholamine release. Paroxysmal or constant **hypertension** is the most classic symptom. Also, sweating, headache, arrhythmias, palpitations, and nervousness may be seen in any combination.
   c. **Laboratory values** show **elevated urinary catecholamines and catacholamine metabolites**.
4. **Neuroblastoma is the most common malignant extracranial solid tumor of childhood**.
   a. **Clinical features.** Tumors grow rapidly, metastasize widely (especially to bone), and produce elevated urinary catecholamines.
   b. **Pathology.** Neuroblastoma occurs most frequently in the adrenal medulla but may also arise in the cervical, abdominal, and thoracic sympathetic chain.

THYROID GLAND

A. **Overview of hyperthyroidism**

1. **Etiology.** Hyperthyroidism may be seen most often in Graves' disease, toxic multinodular goiter, and toxic adenoma. Thyroiditis, thyroid carcinoma, and iodine ingestion are less frequent causes.
2. **Pathogenesis** is due to increased circulating levels of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4), causing a **hypermetabolic state.**

CLINICAL CORRELATION

Clinical diagnosis of hyperthyroidism may be difficult in pregnancy, which is an intrinsically hypermetabolic state and is often associated with mild degrees of thyromegaly. In addition, the increase in TBG that results from the high estrogen levels elevates the total serum T_4, but not the free serum T_4.

3. **Clinical features**
   a. Cardiac symptoms include tachycardia, cardiac palpitations, cardiomegaly, occasional cardiac arrhythmias (usually atrial fibrillation), and cardiomyopathy.
   b. The skin is warm, flushed, and moist due to vasodilatation.
   c. The eyes show a wide stare with upper lid retraction and lid lag. **Exophthalmos** is characteristic of Graves' disease, due to swelling of extraocular muscles and periorbital tissues.
   d. Patients also show increased sweating, heat intolerance, hyperactivity, nervousness, tremor, weight loss, diarrhea, oligomenorrhea, and myopathy.
4. **Diagnosis** is based on increased T_4 and suppressed TSH measurements.

B. **Overview of hypothyroidism**

1. **Etiologies**
   a. Congenital thyroid dysplasia or hypoplasia
   b. Hypothalamic or pituitary disease
   c. Thyroid conditions causing goiter including iodine deficiency and Hashimoto's (autoimmune) thyroiditis
   d. Surgical or radiation destruction of gland
   e. Peripheral resistance to thyroid hormone
2. **Clinical features** depend on the age group.
   a. **Infants** lacking sufficient thyroid hormone develop **cretinism.** The major effects are on skeletal and CNS development (i.e., short stature, retarded bone age, epiphyseal dysgenesis, and mental retardation). Once apparent, the syndrome is irreversible. The initial presentation includes failure to thrive, feeding difficulties, constipation, and somnolence. Children develop protuberant abdomens, wide-set eyes, dry rough skin, broad nose, and delayed epiphyseal closure. Neonatal screening for elevated TSH is essential for early detection.
   b. **Older children** show short stature, retarded linear growth, and delayed onset of puberty
   c. **In adults**, hypothyroidism causes lethargy, weakness, fatigue, decreased appetite, weight gain, cold intolerance, hair loss, dry skin, constipation, apathy, myopathy, psychosis, metrorrhagia (irregular uterine bleeding), and accelerated atherosclerosis with elevated serum cholesterol. **Myxedema,** a syndrome associated with severe hypothyroidism, shows periorbital puffiness, pale doughy skin due to accumulation of hydrophilic mucopolysaccharides, sparse hair, cardiac enlargement, cardiomyopathy, pleural effusions, anemia, and thickened facial features.

NOTE

In contrast to primary hypothyroidism, secondary (pituitary gland failure) and tertiary (hypothalmic failure) hypothyroidism, have low (or normal) TSH levels.

Diagnosis of primary hypothyroidism is based on an elevated TSH and low T_4.

Graves' disease

Incidence. Graves' disease peaks in the third and fourth decades and is five times more common in women. There is a familial predisposition, and it is associated with other autoimmune diseases, such as pernicious anemia and Hashimoto's thyroiditis.

Pathogenesis is **autoimmune,** resulting from production of **thyroid-stimulating immunoglobulin** (TSI) and **thyroid growth immunoglobulin**, two autoantibodies that cause glandular hyperplasia and hormone production by binding to TSH receptors.

. **Clinical features** are present in varying combinations.
   a. **Thyrotoxicosis** has symmetric glandular enlargement.
   b. **Ophthalmopathy** is characterized by lid lag, retraction of the upper lid, proptosis, periorbital edema, and stare.
   c. **Dermopathy** is characterized by thickened edematous nodules or plaques on the lower extremities.

Hashimoto's thyroiditis is a chronic lymphocytic thyroiditis featuring **goitrous** enlargement of the thyroid gland produced by **lymphocytic** and **plasma cell infiltrates**, with the eventual development of hypothyroidism.

1. **Etiology** is **autoimmune**. There may be autoantibodies to the TSH receptor, T_3, T_4, microsomes, and thyroglobulin.
2. **Incidence**. Hashimoto's thyroiditis is the most common type of thyroiditis and is the **leading cause of goitrous hypothyroidism** in the U.S. The highest incidence is in **middle-aged women**, and there is a higher incidence in patients with a family history of Hashimoto's or other autoimmune diseases (e.g., Graves' disease, Sjögren's syndrome, systemic lupus erythematosus).
3. **Clinical features** include painless goiter. Hypothyroidism develops, along with malaise, fever, a decreased T_4, and elevated TSH.

E. **Diffuse nontoxic goiter** is used to describe diffuse enlargement of the gland in **euthyroid patients**.

1. **Incidence**
   a. **Endemic goiters** have a high incidence in certain geographic regions (e.g., mountainous regions or regions far from the ocean). They are caused by **iodine-deficient diets** or increased intake of **goitrogens** (e.g., calcium, fluorides).
   b. **Sporadic simple goiter** is less common. The incidence in women is much greater than in men.

IN A NUTSHELL

Hyperthyroidism *(↓ TSH, ↑ T_3, ↑ T_4)*	***Hypothyroidism*** *(↑ TSH, ↓ T_3, ↓ T_4)*
• *↑ HR*	• *Cretinism in children*
• *Skin moist and flushed*	• *Lethargy in adults*
• *Lid lag*	• *Fatigue*
• *Sweating*	• *Weight gain*
• *Heat intolerance*	• *Cold intolerance*
• *Weight loss*	

F. **Multinodular goiter** develops from chronic diffuse goiters; it may be toxic or nontoxic and may become very large (Figure 4-2).

1. **Clinical features.** Glandular enlargement may cause stridor, dysphagia, and even superior vena cava syndrome (mass effect). Fifty percent produce thyrotoxicosis. These tumors must be differentiated from thyroid cancer, particularly asymmetric tumors in euthyroid patients.

G. **Congenital thyroid conditions**

1. **Agenesis or dysgenesis** are frequent causes of cretinism.
2. **Thyroglossal duct or cyst** may communicate with the skin or base of the tongue. It is formed from nests of incompletely descended midline thyroid tissue.
3. **Ectopic thyroid nests** are usually at the base of the tongue. Prior to removal, it must be documented that the patient has other functioning thyroid tissue.

H. **Tumors.** Thyroid nodules are very common (4%-7% adults in the U.S.), but thyroid cancer is uncommon (less than 2 cases per 1000 nodules). There is a higher incidence of neoplasia in solitary nodules and in younger patients.

1. **Adenomas. Follicular adenoma** is the most common.
   a. **Clinical features.** Adenomas may cause pressure symptoms, pain, and, rarely, thyrotoxicosis.
2. **Cysts** make up 10%-25% of solitary nodules and usually represent cystic degeneration of follicular adenomas.

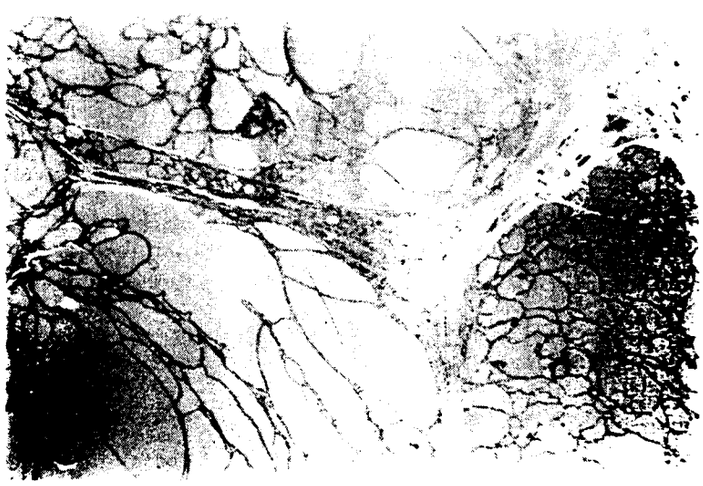


Figure 4-2. Thyroid: multinodular goiter, microscopic.

3. **Carcinomas** represent neoplasia of **follicular cells** (i.e., papillary, follicular, or anaplastic cancer), **parafollicular cells** (i.e., medullary cancer), or **connective tissue.** Risk factors include radiation and a genetic predisposition.
   a. **Papillary carcinoma** is the most common type. The incidence is higher in women.
   b. **Follicular carcinoma** makes up 20% of thyroid cancers and is more malignant than papillary cancer.

PARATHYROID GLANDS

A. Primary hyperparathyroidism

1. **Etiology**
   a. **Parathyroid adenoma** is the most common cause, usually involving a single gland.
   b. **Parathyroid hyperplasia** shows diffuse enlargement of four glands, usually composed of chief cells.
2. **Clinical features.** Patients with **elevated serum calcium** are often asymptomatic. They may present with bone abnormalities secondary to elevated parathyroid hormone (e.g., osteomalacia, osteitis fibrosa cystica, subperiosteal resorption). Hypercalcemia may cause metastatic calcification (e.g, kidney stones).

B. Secondary hyperparathyroidism

1. **Etiology.** Secondary hyperparathyroidism is usually caused by **chronic renal failure,** leading to decreased Ca^{2+} absorption, which in turn results in a feedback loop and increased PTH. Vitamin D deficiency and malabsorption are less common causes.
2. **Clinical features** show soft tissue calcification and osteosclerosis. Mild-to-moderate hypocalcemia is characteristic.

C. Hypoparathyroidism

1. **Etiology.** Common causes are removal of glands during thyroidectomy, idiopathic, radioactive iodine therapy for Graves' disease, metastatic cancer, and DiGeorge's syndrome. The idiopathic form may be familial and autoimmune.
2. **Clinical features** include hypocalcemia, hyperphosphatemia, irritability, anxiety, neuromuscular excitability, tetany, intracranial calcifications, lens calcification, dental abnormalities, and cardiac conduction defects.

D. Pseudohypoparathyroidism

1. **Etiology.** Pseudohypoparathyroidism is an autosomal recessive disorder resulting in a kidney unresponsive to circulating PTH.

IN A NUTSHELL

- *Papillary carcinoma → lymph node metastases*
- *Follicular carcinoma → hematogenous metastases*

NOTE

Lab abnormalities in 1° hyperparathyroidism:

- *↑ PTH*
- *↑ Ca^{2+}*
- *↓ Phosphate*
- *↑ Alkaline phosphatase*

CLINICAL CORRELATE

Osteitis fibrosa cystica, also known as von Recklinghausen's disease of bone, occurs as a result of chronic primary hyperparathyroidism. Cystic changes in bone occur due to osteoclastic resorption. Fibrous replacement of resorbed bone may lead to a "brown tumor," a non-neoplastic tumor mass.

NOTE

Lab abnormalities in 2° hyperparathyroidism:

- *↑ PTH*
- *↓ Ca^{2+}*
- *↑ Phosphate*
- *↑ Alkaline phosphatase*

CLINICAL CORRELATE

DiGeorge's syndrome is also associated with absence of the thymus due to a common embryologic defect. Tetany occurs shortly after birth due to congenital absence of the parathyroid glands. Cardiac structural defects and immunodeficiency are also noted.

2. **Clinical features** include skeletal abnormalities such as short stature, and shortened fourth and fifth carpals and metacarpals.

E. **Hypercalcemia** is defined as a persistent serum calcium over 10.4 mg/dl.

1. **Etiology.** Hypercalcemia may be caused by metastatic disease to bone, such as myeloma or epithelial neoplasm, vitamin D intoxication, sarcoidosis, primary or secondary hyperparathyroidism, the milk alkali syndrome, or Paget's disease of bone.
2. **Clinical features. Renal stones** are often seen; hyperparathyroidism is also usually associated with **hypercalciuria** and with **hypophosphatemia**. Alkaline phosphatase activity is usually elevated. Patients may experience an altered sensorium, often first noticed as drowsiness.
3. **Pathologic features** in bone range from obvious metastases to osteoclast tunneling through bony trabeculae in hyperparathyroidism.

MNEMONIC

Hypercalcemia:
***M**alignancy*
***I**ntoxication*
***S**arcoidosis*
***H**yperparathyroidism*
***A**lkali syndrome*
***P**aget's disease*

ENDOCRINE PANCREAS (Islets of Langerhans)

A. **Diabetes mellitus** is caused by inadequate or abnormal insulin secretion, causing impaired glucose utilization, and resulting in hyperglycemia, glycosuria, and characteristic systemic pathology.

1. **Types**
   a. **Insulin-dependent (type I) diabetes mellitus (IDDM).** There is an abrupt onset with patients prone to **ketoacidosis,** insulin dependence, and severe metabolic derangements.
   b. **Noninsulin-dependent (type II) diabetes mellitus (NIDDM).** This disease constitutes most cases of idiopathic diabetics. It is characterized by an abnormality of insulin secretion or peripheral insulin resistance. Most patients have central obesity with an onset of disease usually after age 40. These patients are not prone to ketoacidosis.
   c. **Secondary diabetes** may be caused by destruction of pancreatic islet cells from inflammation, hemochromatosis, tumor, surgery, or hormonal disease.
2. **Pathogenesis**
   a. **IDDM** shows a marked, absolute insulin deficiency resulting from **diminished β-cell mass**. It it therefore characterized by **low serum insulin levels**. There are three etiologic theories; in many cases of IDDM, all three mechanisms may be operative.

(1) A **viral infection** (e.g., mumps, coxsackievirus B, rubella, CMV, mononucleosis) may lead to destruction of β cells. .
(2) There is clearly a **genetic predisposition**.
(3) **Autoimmune response.** Eighty percent of patients with IDDM have **anti-islet cell antibodies**.

b. **NIDDM** is characterized by mild-to-moderate insulin deficiency and is not associated with a specific HLA haplotype. There are two theories:
(1) Delayed or inadequate insulin secretion may develop for unknown reasons.
(2) Insulin resistance, the impaired ability of tissues to react to circulating insulin, results from a decrease in the number of cell-surface insulin receptors, again for unknown reasons.

3. **Clinical features**
a. **Predisposing factors** are obesity, pregnancy, trauma, infections, and stress.
b. **Presentation.** Both IDDM and NIDDM may present with **polydipsia**, **polyuria**, **polyphagia**, **weight loss**, and muscle weakness. Laboratory values may show **hyperglycemia**, **glycosuria**, and **hyperlipidemia**.
c. **Acute metabolic complications**
(1) **Diabetic ketoacidosis (DKA)** may occur in insulin-dependent diabetics. It leads to an oversupply of glucose, fueled by high rates of protein catabolism, lipolysis in adipose tissue, and fatty acid oxidation in liver. The accelerated rate of fatty acid oxidation produces acetyl-CoA faster than it can be burned by the TCA cycle, and the liver conserves the excess acetyl-CoA by synthesizing ketones. Metabolic acidosis results from the accumulation of the ketones. The high level of blood glucose leads to dehydration via an osmotic diuresis. Treatment with insulin normalizes the metabolism of carbohydrate, protein, and fat. Fluids are given to correct the dehydration.
(2) **Hyperosmolar nonketotic coma** occurs in patients with mild adult-onset diabetes when blood glucose levels exceed approximately 600 mg/dl.
d. **Late complications of diabetes.** Patients with long-standing diabetes of either type may develop a series of long-term complications.
(1) **Atherosclerosis** causes strokes, myocardial infarcts, and gangrene, frequently of the toes.
(2) **Nephropathy** causes proteinuria, hypertension, and edema, and it may lead to renal failure.

CLINICAL CORRELATE

Diabetics are also a high-risk group for the following infections:

- *Klebsiella pneumonia*
- *Sinus mucormycosis*
- *Malignant otitis externa (Pseudomonas aeruginosa)*
- *Chronic osteomyelitis*

(3) In the **Kimmelstiel-Wilson syndrome**, intercapillary glomerulosclerosis with hypertension and edema lead to proteinuria, beginning approximately 20 years after the onset of disease.

(4) There is a predisposition to **infections** (tuberculosis, pyelonephritis, pneumonia, skin infections).

(5) **Neuropathy** is usually a distal, symmetric polyneuropathy ("stocking-glove" distribution) but may be a mononeuropathy. In addition to this **peripheral neuropathy**, diabetics can also have **autonomic neuropathy**.

(6) **Retinopathy** may lead to blindness.

4. **Prognosis**
   a. **NIDDM** decreases life span by approximately 8 years. There is a much higher mortality from IDDM.
   b. **Causes of death** in decreasing frequency are:
      (1) Myocardial infarction
      (2) Renal failure
      (3) Stroke
      (4) Ischemic heart disease
      (5) Infections

B. **Islet-cell tumors**

1. **β-cell tumors. Insulinomas** most commonly occur between the ages of 30 and 60.
   a. **Pathogenesis.** β-cell tumors produce hyperinsulinemia, causing **hypoglycemia.**
   b. **Clinical features.** Patients experience episodes of altered sensorium (i.e., disorientation, dizziness, diaphoresis, nausea, tremulousness, coma) that are relieved by glucose intake.
   c. **Pathology.** Most tumors are solitary, well-encapsulated, and well-differentiated adenomas of various sizes. Ten percent are malignant carcinomas.
2. **Zollinger-Ellison syndrome** is due to a gastrinoma and is often associated with MEN type I.
   a. **Pathogenesis.** Tumors of pancreatic islet cells secrete gastrin, causing gastric hypersecretion of acid.
   b. **Clinical features** include **intractable peptic ulcer disease** and severe diarrhea.
   c. **Pathology.** Sixty percent are malignant. Most tumors are located in the pancreas, with 10% in the duodenum.

BRIDGE TO GASTROINTESTINAL

Zollinger-Ellison syndrome is discussed in greater detail in the Gastrointestinal Pathology chapter of this book.

Respiratory Pathology

The lung is a major destination for anything that can float in the air, including pollutants, spores, bacteria, viruses, and smoke. As a result, it is a primary site for inflammation, infection, and neoplasia.

Lung cancer is now the leading cause of cancer death in both men and women; approximately 90% of cases are due to cigarette smoking. In additon, common allergic and destructive inflammatory conditions, such as asthma, bronchitis, and emphysemas, are seriously exacerbated by smoking. This chapter will discuss the different pulmonary pathologies associated with infectious and neoplastic diseases, as well as the common environmental agents known to cause and/or exacerbate pulmonary disorders.

CONGENITAL ANOMALIES

A. **Pulmonary cysts.** There are two types of pulmonary cysts caused by premature separation of the embryonic foregut.

1. **Bronchogenic cysts** are centrally located, adjacent to bronchi or bronchioles, and occur with or without connections to airways. They are lined by ciliated, mucus-secreting bronchial columnar epithelium and may be single or multiple. Their size varies from microscopic to greater than 5 cm in diameter, and they may be associated with other cysts of the liver, kidney, or pancreas.
2. **Pulmonary cysts** are multiple and peripherally located, lacking communication with main bronchi. Infection is frequent (e.g., abscess); rupture can cause pneumothorax and compression of adjacent lung tissue. Dilatation may rupture vessels, leading to hemoptysis.

BRIDGE TO GASTROINTESTINAL

Neonates with either esophageal atresia or tracheoesophageal fistula are vulnerable to aspiration pneumonia.

B. **Pulmonary atresia.** Bilateral pulmonary atresia is not compatible with life; unilateral atresia is usually accompanied by other serious malformations.

C. **Pulmonary hypoplasia** refers to incomplete development of the entire lung or a single lobe of the lung.

D. **Congenital lobar overinflammation** results from bronchial obstruction due to absence or hypoplasia of the bronchial cartilage with compensatory overinflation of the remaining lung.

E. **Pulmonary sequestrations.** Extrapulmonary lung tissue is usually supplied by systemic blood vessels rather than by pulmonary arteries. It is usually located behind the lung or below the diaphragm.

INFECTIONS

NOTE

The spectrum of infectious agents causing pneumonia continues to change as antibiotics evolve and the number of immunocompromised patients rises.

Infections in the lung are more common than infections in any other organ; viral infections are more frequent than other forms of pulmonary infection.

A. **Bacterial pneumonia** occurs when pulmonary defense mechanisms are weakened (e.g., decreased cough, gag, or nasal clearance; mucociliary damage; macrophage phagocytic defects; pulmonary edema; pooling of secretions; bronchial injury) or when the host is otherwise immunocompromised (e.g., chronic disease, immunologic deficiency, immunosuppressive therapy, leukopenia). It can be classified in several ways:

   1. By **etiologic agent** (e.g., staphylococcal, streptococcal)
   2. By **host response** (e.g., suppurative, fibrinous)
   3. By **anatomic distribution** (e.g., bronchopneumonia, lobar pneumonia, interstitial pneumonia)

B. **Bronchopneumonia** causes a patchy consolidation of the lung and usually arises as an extension of pre-existing bronchitis or bronchiolitis.

   1. **Incidence.** It occurs most commonly in infancy and old age. The most common agents include *Streptococcus pneumoniae,* Staphylococcus, *Haemophilus influenzae,* Pseudomonas, and coliforms. Fungi may be pathogenic in immunosuppressed hosts.
   2. **Clinical features** include fever, a cough productive of purulent sputum, rales over involved areas, and pleuritic chest pain if peripheral regions are involved. Chest x-ray shows focal opacities.

3. **Pathology**
   a. **Grossly,** up to 3–4 cm foci of lung consolidation with purulent inflammation are seen. Consolidation is frequently multilobar, bilateral, and basal because of gravitational pooling of the infection.
   b. **Microscopic findings** are usually a purulent exudate, dominated by neutrophils filling airways and alveoli (see Figure 5-1), unless the patient is immunosuppressed.
4. **Complications** include lung abscess, spread to the pleural space (empyema), spread to the pericardial cavity (suppurative pericarditis), bacteremia with metastatic infection, and respiratory failure.

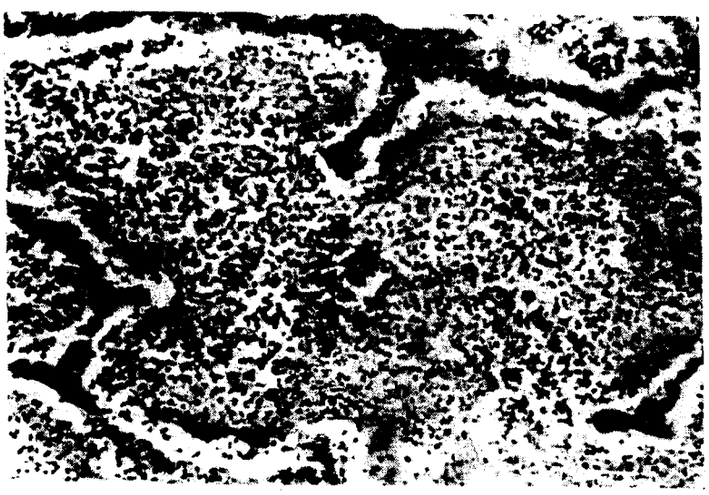


Figure 5-1. Acute bronchopneumonia (microscopic).

C. **Lobar pneumonia** is usually due to a bacterial infection, most commonly caused by *S. pneumoniae,* leading to widespread consolidation in large portions of a lobe.
   1. **Incidence.** Lobar pneumonia occurs most often in mid-life. Men are involved 3–4 times more frequently than women. Klebsiella and type II pneumococcus occur in the elderly, alcoholics, and diabetics.
   2. **Clinical features.** There is an acute onset of fever, chills, malaise, and cough with watery sputum initially, followed by frankly purulent, **rusty sputum.** Shortness of breath, orthopnea, and cyanosis can occur if pneumonia is sufficiently severe. Pleuritic chest pain and pleural friction rub occur with peripheral involvement. Limited breath sounds and rales occur early, proceeding to dullness and percussion with egophony.

IN A NUTSHELL

Bronchopneumonia:

- *Patchy consolidation involving one or more lobes*
- *Acute inflammation (neutrophils) extending into alveoli from bronchioles*

BRIDGE TO MICROBIOLOGY

Pneumonia in diabetics or alcoholics → think Klebsiella. Another classic clue for Klebsiella pneumonia is "currant jelly" sputum.

Increased tactile and vocal fremitus occur with more severe consolidation. Chest x-ray shows lobar involvement.

3. **Complications** include lung abscess, empyema, and exudate organization rather than resorption. This causes respiratory difficulty and bacteremia, with metastases to heart valves (endocarditis), spleen, brain (meningitis), kidney, joints, and pericardium (pericarditis).

D. **Viral and mycoplasmal pneumonia (atypical pneumonia).** These reactions are called atypical because of lack of alveolar exudate. Instead, inflammation is found in the lung interstitium and alveolar septae **(interstitial pneumonia)**. Pneumonia is frequently caused by *Mycoplasma pneumoniae* in crowded conditions and by viruses, including influenza A and B, respiratory syncytial virus (RSV), and rhinovirus.

1. **Clinical features** include fever, malaise, and a **dry hacking cough;** these symptoms resemble those of a severe upper respiratory infection. Constitutional symptoms are common: headache, muscle aches, and leg pains. **Elevated cold agglutinins** are found in 50% of patients with mycoplasmal pneumonia and 20% of patients with adenovirus. There is less than 1% mortality. Symptoms are out of proportion to physical findings.
2. **Pathology**
   a. **Grossly,** there is patchy-to-diffuse involvement, bilaterally or unilaterally. Affected areas are red-blue with a congested interstitium but without consolidation or pleural involvement. There is no pus.

E. ***Pneumocystis carinii* pneumonia (PCP).** *Pneumocystis carinii* is now believed to be a fungal organism; it infects immunocompromised patients. It is commonly seen in **acquired immunodeficiency syndrome (AIDS),** in oncology patients, as well as in undernourished children.

1. **Clinical features**. Patients present with fever, dyspnea (shortness of breath), hypoxia (low oxygen saturation), and bilateral interstitial infiltrate on x-ray. Less often, patients complain of cough.

F. **Aspiration pneumonia** results from aspiration of oral secretions or gastric contents. It is seen in **alcoholics and other debilitated patients** with neurologic or anatomic impairment affecting the swallowing mechanism. A chemical pneumonitis results, often with secondary bacterial infection from mouth anaerobes, causing necrosis and abscess formation.

IN A NUTSHELL

Lobar pneumonia:

- *Called "lobar" because it involves the entire lobe*
- *Most often due to Streptococcus pneumoniae*
- *Characterized mainly by an intra-alveolar exudate that results in consolidated lobes(s) of the lung*
- *Red hepatization followed by grey hepatization*

IN A NUTSHELL

Interstitial pneumonia:

- *Most commonly due to viruses and Mycoplasma pneumoniae*
- *Inflammation is found in the lung interstitium and alveolar septae; there is no alveolar exudate*
- *Involves one or more lobes*

BRIDGE TO MICROBIOLOGY

The other fungal diseases affecting the respiratory tract (e.g., histoplasmosis, coccidioidomycosis, blastomycosis, aspergillosis) are discussed in detail in the Mycology chapter of the Microbiology review notes.

G. **Pulmonary abscess** refers to an area of inflammation with a central region of liquefaction necrosis. It occurs at any age but is more common in young adults (men > women). It is rare in infants.

1. **Pathogens** include aerobic and anaerobic Streptococcus, *S. aureus*, Gram-negative rods, and mouth anaerobes, including Bacteroides, Fusobacterium, and Peptostreptococcus.
2. **Routes of infection**
   a. **Aspiration** of gastric contents and mouth flora
   b. Bacterial pneumonia **(inhalation)**
   c. Septic **emboli** from the venous circulation or the right side of the heart
   d. **Neoplasia** with postobstructive pneumonia
   e. Miscellaneous **trauma**, extension of infection from other organs, hematogenous spread, or cryptogenic (no identifiable cause)
3. **Clinical features** include fever, paroxysmal cough with **foul-smelling,** purulent or sanguineous sputum, and weight loss. Clubbing can occur within weeks of abscess formation. 10-15% have underlying carcinoma. With appropriate antibiotics, 75% of pulmonary abscesses resolve without sequelae. An **air-fluid level** is often seen on chest x-ray.
4. **Complications** include respiratory failure, extension of infection into the pleural space, and embolization to the brain and meninges.

H. **Pulmonary tuberculosis** (TB) primarily affects the lungs and is caused by **acid-fast mycobacteria.** Almost all cases are caused by *Mycobacterium tuberculosis*. Atypical mycobacteria can cause infection, especially in the immunocompromised host. Because *M. tuberculosis* is a **strict aerobe**, reactivation tends to occur in the apex of the lung and renal cortex. There is an increased incidence in areas with poor sanitary conditions, poverty, overcrowding, malnutrition, and limited access to medical care. The emergence of AIDS and other immunosuppressed states has led to a resurgence in the incidence of TB. Of concern now is the occurrence of **multiple drug-resistant** TB.

1. **Primary pulmonary TB**
   a. **Pathology.** The lung is the usual location of initial infection, typically the lower part of the upper lobe or the upper part of the lower lobe. Parenchymal or subpleural lesions occur associated with enlarged, ipsilateral caseous lymph nodes, which are "draining" the parenchyma. The **"Ghon complex"** refers to radiographic evidence of a calcified peripheral lesion in conjunction with a calcified hilar lymph node.

b. **Clinical features**. Most patients are asymptomatic, and the lesions become fibrotic and calcified over time. It is the macrophage that leads to phagocytosis of tubercle bacilli, epitheloid giant cell fusion, and **granuloma formation** with central **caseous necrosis**. The tubercle bacilli survive in granulomas for years, only to reactivate when the patient's immune system is depressed (e.g., elderly, malnourished, HIV).

2. **Secondary pulmonary TB**. Most cases represent **reactivation** (rather than reinfection) of old TB that had disseminated at the time of primary TB. Reactivation occurs often in areas of high oxygen tension, such as the **lung apices.** Only 5%–10% of patients exposed to TB develop reactivation. Reactivation TB usually occurs in debilitated elderly patients.
   a. **Pathology**
      (1) **Grossly,** there is a small focus of consolidation, usually less than 3 cm, in the lung apex. Hilar lymph nodes are also involved, developing foci of tuberculous activity. Parenchymal lesions can develop small areas of caseous necrosis that may not cavitate. The usual course is fibrous encapsulation, leading to fibrocalcific scars and pleural adhesions. A thick, collagenous wall may totally enclose caseous debris. This may never resolve and can remain as a granular lesion.
      (2) **Microscopically,** characteristic **granulomas** composed of epithelioid cells, with occasional Langhans' giant cells, are seen.
   b. **Complications** include hemoptysis, resulting from ulceration of the bronchial mucosa; pleuritis, tuberculous pneumonia, and bronchopleural fistula with empyema.
3. **Late progressive pulmonary TB** shows progression of an early tuberculous apical lesion to a fibrocaseous area with cavitation. Spread is through erosion into an airway to other regions of the lung, resulting in multiple lesions that may cavitate. Spread may also occur via the lymphatic system or blood, leading to distant dissemination. The pleura is often involved and may lead to exudative pleural effusion, frank tuberculous empyema, or massive obliterative fibrous pleuritis. Bronchi are also involved as a result of seeding and can cause mucosal ulcers. Pathology reveals caseating granulomas.
4. **Miliary TB** is due to spread via blood or lymphatics. Disease may remain confined to the lung but usually disseminates widely. For example, erosion into a pulmonary artery leads to lung lesions; erosion into a pulmonary vein leads to systemic lesions. Extrapulmonary sites of involvement include the renal

cortex, lymph nodes, genital tract, peritoneum, bone marrow, adrenal gland, pericardium, and meninges.

Legionella infections. *Legionella pneumophila* (a Gram-negative bacillus) is the etiologic agent of these infections. It is usually found in soil or water. Transmission is via **inhalation** into the lungs. Major environmental sources include **water reservoirs and cooling units** of air conditioning systems that may contain blue-green algae and amoebae, among which Legionella can survive for prolonged periods.

1. **Clinical features**. Community outbreaks traced to an infected water source reveal two patterns of illness.
   a. **Pontiac fever** is a mild, nonfatal, systemic febrile illness.
   b. **Legionnaire's disease** is a severe pneumonia with 15%–20% mortality. After approximately 5 days of incubation, patients develop fever, dry cough, malaise, chest and abdominal discomfort, confusion, and occasionally, diarrhea. Frequently, pulse-temperature dissociation exists (a high temperature with no increase in pulse). Severe cases have blood-tinged sputum, dyspnea, high fevers, and impressive systemic symptoms. Death may occur due to progressive ventilatory failure or from a shock-like syndrome with disseminated intravascular coagulation (DIC) and renal failure.
2. **Complications**
   a. Inflammation of small pulmonary arteries and veins can lead to thrombosis.
   b. Abscess formation is frequent, but the abscesses are small.
   c. Organization and scarring secondary to destructive lesions can lead to ventilatory impairment.
   d. Fibrinous pleuritis is usually mild with serous effusion.
   e. Bacteremia is always a risk.

J. **Diphtheria** (due to *C. diphtheriae*) and **whooping cough** (due to *B. pertussis*) both cause toxin-mediated upper respiratory tract infections that can be accompanied by lower respiratory tract infection. The diphtheria toxin induces necrosis of the epithelium of the upper respiratory tract, resulting in the formation of a "diphtheric pseudomembrane."

CLINICAL CORRELATE

Legionella pneumophila infection is a result of inhalation of the aerosol from contaminated water, most commonly found in air conditioning systems.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

COPD is a group of disorders characterized by **increased resistance to airflow** during both inspiration and expiration, due to airway obstruction. The obstruction can occur at any level from the trachea to terminal bronchioles. This group represents the most common

NOTE

COPD is a group of disorders that includes:

- *Emphysema*
- *Chronic bronchitis*
- *Asthma*
- *Bronchiectasis*

form of pulmonary disease and includes emphysema, chronic bronchitis, asthma, and bronchiectasis.

A. **Emphysema** refers to distention of air spaces distal to the terminal bronchiole with destruction of alveolar septae, probably secondary to ischemia.

1. **Incidence.** Emphysema is associated with **cigarette smoking,** urban living, and pollution. Cigarette smoke causes an increase in elastase availability (released by neutrophils and macrophages) and a decrease in antielastase activity (due to oxidant effects). Men are affected more frequently than women.

2. **Types**

a. **Centrilobular emphysema** affects the **central and proximal** part of a lobule; distal alveoli are not involved. It is more common and usually more severe in the upper lobes. Inflammation surrounding bronchi, bronchioles, and alveoli is common.

b. **Panacinar emphysema** causes a uniform enlargement of lobules, including terminal and respiratory bronchioles as well as distal alveoli. It is more common and more severe in the lower lobes. **Alpha$_1$-antitrypsin deficiency** is thought to lead to an imbalance between protease and antiprotease activity. This imbalance then leads to panacinar emphysema by young adulthood, especially in the lower lungs.

c. **Paraseptal emphysema** involves the **distal region of the acinus**, sparing terminal bronchioles and respiratory bronchioles. It is most severe along the pleura, septae, and the lobule edge. It commonly occurs adjacent to areas of fibrosis, scarring, or atelectasis, and is more severe in the upper lung. Paraseptal emphysema forms multiple confluent distended air spaces. It may be the cause of spontaneous pneumothorax (collapsed lung) in young adults.

d. **Irregular emphysema** describes irregular acinus involvement. It is associated with scarring.

e. **Bullous emphysema** refers to large, balloon-like distended air spaces in the lung periphery, which can lead to pneumothorax.

f. In **interstitial emphysema,** an alveolar tear allows air into the connective tissue stroma of the lung, mediastinum, or subcutaneous tissue.

3. **Pathology**

a. **Centrilobular emphysema** (Figure 5-2)

NOTE

- *α_1-antitrypsin inhibits the destruction of elastin by elastase, a proteolytic enzyme carried by inflammatory cells. Elastase acts on alveolar walls.*
- *α_1-antitrypsin deficiency is a hereditary disorder that results in defective secretion of α_1-antitrypsin by the liver. In homozygotes, this eventually results in panacinar emphysema and hepatic cirrhosis.*

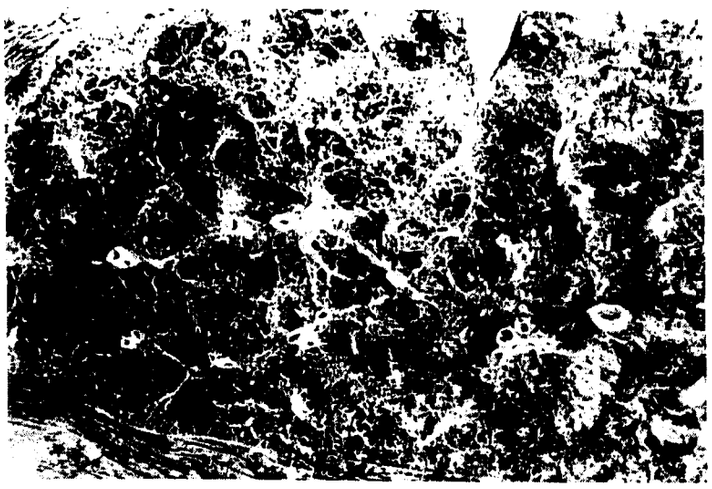


Figure 5-2. Centrilobular emphysema (gross).

(1) **Grossly,** the lungs may not be particularly enlarged or pale unless disease is well-advanced. The upper two-thirds are more severely involved.

(2) **Microscopically**, central airspaces (respiratory bronchioles and alveolar ducts) are destroyed with sparing of peripheral alveoli; inflammation around bronchi and bronchioles is common.

b. **Panacinar emphysema**

(1) **Grossly,** panacinar emphysema causes hyperinflated lungs with increased crepitance. Involved areas are pale as a result of blood vessel destruction and compression.

(2) **Microscopically**, there is little inflammatory involvement of septae or alveoli associated with their destruction.

4. **Clinical features** include dyspnea with or without cough; weight loss; **barrel-chest** due to hyperinflation; pursed-lip breathing; prolonged expiratory time, and **cor pulmonale** (right-sided heart failure). "**Pink puffers**" are patients who overventilate to maintain oxygenation despite the elevated work of breathing. X-rays reveal **hyperinflation** with flattened diaphragms.

5. **Pathogenesis.** There are two theories.

a. **Protease-antiprotease theory**, as described above.

b. Loss of bronchial cilia as a result of smoking leads to mucus plugging and **alveolar overdistension**. Alveolar overdistention, resulting from obstruction, can compromise the septal blood flow, leading to ischemia and alveolar destruction. Inflammation and mucus plugging may exacerbate the obstruction.

6. **Complications** include **cor pulmonale** as a result of increased pulmonary vascular resistance, ventilatory failure, polycythemia, and pneumothorax.

B. **Chronic bronchitis** is a common disorder that can lead to obstructive airway disease. Chronic bronchitis is a **clinical diagnosis**, that is, persistent cough with sputum production for at least 3 months for 2 consecutive years. Sputum varies from uninfected mucus (simple chronic bronchitis) to purulent (mucopurulent chronic bronchitis).

1. **Pathogenesis.** There are two major factors.
   a. **Chronic irritation** from inhaled substances (e.g., nitrogen dioxide, sulfur dioxide) may cause inflammation.
   b. **Recurrent infections** do not initiate bronchitis, but they do perpetuate it, and result in acute exacerbations. Common organisms include *Haemophilus influenzae*, *S. viridans*, and *S. pneumoniae*. Smoking can lead to both irritation and infection. Smoke destroys the lung's ciliary tree, damages the mucosa, and interferes with WBC function. It is believed that changes in the small airways are important in the pathogenesis of bronchitis. Small airway obstruction represents the earliest manifestation of COPD. Inflammation and mucus plugging increase resistance to air flow in these usually low-resistance airways. Continued exposure to irritants and repeated infection eventually lead to chronic bronchitis.

2. **Pathology**
   a. **Grossly**, lungs are boggy, hyperemic, and hyperinflated with **copious mucus plugging** the airways.
   b. **Microscopically**, there is hypertrophy of the submucosal glands first in the large airways then in smaller airways. Bronchial epithelium may exhibit squamous metaplasia or dysplasia. Mucus plugging, inflammation, edema, smooth muscle hypertrophy, and fibrosis are all common.

3. **Clinical features**. There is a **productive cough** with copious sputum production, dyspnea, barrel chest, cyanosis, hypercapnia, hypoxia, and frequent infection. Patients are classically known as "**blue bloaters,**" because they are constantly cyanotic.

4. **Complications. Respiratory failure** usually occurs during a bout with an acute infection. **Cor pulmonale** may occur as a result of pulmonary hypertension (increased resistance of pulmonary vasculature as a result of alveolar destruction and hypoxic vasoconstriction). Dysplasia of bronchial epithelium may lead to cancer.

IN A NUTSHELL

Chronic bronchitis:

- *Is a clinical diagnosis of persistent cough with sputum production for at least 3 months for 2 consecutive years*
- *Is associated with infections, cigarette smoking, air pollution, and various genetic factors*
- *Can present with mucus plugging, inflammation, edema, fibrosis, and smooth muscle atrophy*

C. **Asthma** is characterized by enhanced airway reactivity, leading to intermittent episodes of **reversible paroxysmal airway narrowing.**

1. **Types**
   a. **Extrinsic asthma (allergic, atopic)**. Attacks are triggered by environmental antigens (e.g., dust, pollen, food). There is frequently a family history of **atopy** (e.g., rhinitis, asthma, and eczema). Bronchospasm is mediated by a **type I immunoglobulin E (IgE) hypersensitivity** response to a particular antigen. Histamine, leukotrienes (LTC_4, LTD_4, and LTE_4), prostaglandin D_2 (PGD_2), chemotactic factors, and platelet activation all lead to airway-constricting inflammation and increased vascular permeability. Serum IgE levels are elevated, and a positive skin test may be demonstrated to the offending antigen.
   b. **Intrinsic asthma (idiosyncratic)**. Exacerbations frequently follow a viral infection, which causes inflammation and a lowering of the vagal threshhold for irritants. Other causes of increased airway reactivity include stress, pollution, occupational exposure, exercise, and cold weather. There is no family history, skin tests are negative, and IgE levels are normal.
   c. **Aspirin-induced asthma** may be seen in adults. There is a classic triad of **nasal polyps, rhinitis, and bronchoconstriction.**

2. **Pathology**
   a. **Grossly**, asthma causes hyperinflated lungs with small areas of atelectasis. Bronchi and bronchioles are occluded by thick, tenacious mucus plugs.

3. **Clinical features** include cough, dyspnea, and **wheezing**. X-ray reveals hyperinflation. If airway obstruction is severe, the patient may not be able to ventilate, leading to respiratory failure (increased Pco_2 and decreased Po_2). Between attacks, patients are asymptomatic.

D. **Bronchiectasis** is an abnormal, permanent dilatation of airways, due to **chronic necrotizing infection** and obstruction.

1. **Pathogenesis**
   a. **Bronchial obstruction** (e.g., tumor, foreign body, COPD, mucus plug) leads to atelectasis and airway smooth muscle relaxation.
   b. **Infection** further weakens the airway wall. Organisms include Staphylococcus, Streptococcus, enteric anaerobes, and *H. influenzae*. Patients are susceptible to recurrent infection due to impaired defense against pathogens,

NOTE

In contrast to delayed hypersensitivity skin tests in TB, where the reaction takes 2-3 days to form, the IgE-mediated reaction is referred to as immediate hypersensitivity and produces a wheal and flare in a few minutes.

IN A NUTSHELL

Some microscopic pathologic findings in asthma:

- *Mucus plugs containing Curschmann's spirals and Charcot-Leyden crystals*
- *Eosinophilic infiltrate*
- *Edema*
- *Submucosal gland hypertrophy*
- *Bronchial wall muscle hypertrophy*

caused by cough, injury to the mucociliary apparatus, and impaired phagocytosis.

c. Examples of disorders in which chronic infection leads to bronchiectasis include:

(1) **Cystic fibrosis,** which is characterized by exocrine gland dysfunction, leading to viscous sputum.

(2) **Kartagener's syndrome,** one of several immotile cilia syndromes, is characterized by a triad of **sinusitis, bronchiectasis, and *situs inversus***. Absence of pulmonary cilia interferes with bacterial clearance.

2. **Pathology**

a. **Grossly,** bronchiectasis predominantly affects the lower lobes. Dilated airways may be cylindroid, fusiform, or saccular. The lumen is filled with a purulent exudate and the mucosa is edematous and ulcerated.

3. **Clinical features** include cough, fever, and foul-smelling purulent sputum, which is most copious in the morning due to pooling. Clubbing and frequent pneumonia may also be seen.

4. **Complications** include lung abscess, pneumonia, empyema, and septic emboli.

RESTRICTIVE LUNG DISEASE

This is a group of diseases characterized by **decreased lung compliance,** i.e., stiff lungs. The decreased compliance results in small lung volumes with augmented air flow rates. Varying pathologic processes can result in restriction, including extrinsic disease (neuromuscular, chest wall, myasthenia) and intrinsic lung disease. Intrinsic lung processes include interstitial and infiltrative disease, adult respiratory distress syndrome (ARDS), pneumoconiosis, and granulomatous disease.

A. **Adult respiratory distress syndrome (ARDS)** is the final common pathway of **acute diffuse alveolar damage** (both physiologic and histopathologic). It can be caused by a variety of insults, including sepsis/shock, pancreatitis, burns, trauma, drug overdose, pneumonia, and toxins.

1. **Clinical features** include the rapid onset of severe respiratory insufficiency, resulting from alveolar flooding with impaired ventilation (decreased Po_2; increased Pco_2).

B. **Pneumoconiosis** refers to the presence of environmental "dust" in the lung and the lung's response to this foreign entity. It applies to any aerosol, whether in the form of fumes or particulate matter. Development of disease depends upon the amount of exposure, the size and shape of the particles, and the solubility

and cytotoxicity of the offending material. All can result in progressive massive fibrosis with diffuse scarring and restrictive lung disease.

1. **Coal workers' pneumoconiosis** occurs after prolonged periods (>10 years) of exposure to coal dust containing both carbon and silica.
   a. **Clinical features**. Most are asymptomatic or have a slight cough productive of **blackened sputum.** X-ray reveals diffuse nodularities ("tattooing"). A small number of cases go on to develop progressive disease with dyspnea, chronic cough with blackened sputum, poorly localized chest pain, and frequent infections. If exposure continues, **progressive massive fibrosis** with large blackened scars (usually in the upper regions of various lobes) with cor pulmonale can develop, and the pleura can become retracted and thickened if near fibrotic lesions.
   b. **Pathology. Microscopically, "coal dust macules"** are formed initially by the aggregation of macrophages, creating intensely pigmented areas.
2. **Anthracosis** is due to the inevitable inhalation of some carbonaceous particles by city dwellers, cigarette smokers, and miners.
   a. **Clinical features**. Deposition of **carbon dust** can be seen as black pigment in lung parenchyma, pleura, and lymph nodes. When isolated, it is not associated with symptomatic disease.
   b. **Pathology.** Macrophages aggregate into small, peribronchiole regions in an attempt to phagocytose the dust.
3. **Silicosis.** Chronic silicosis occurs with prolonged exposure to silica dust (mining, glass production, sand blasting, farming, road construction), causing an insidious disease that can progress to respiratory failure and death.
   a. **Clinical features**. Patients with silicosis are at increased risk of developing TB. There is no associated increased cancer risk.
   b. **Pathology.** Collagenous fibrotic nodules form wherever the silica is deposited, probably due to macrophage release of lysosomal enzymes and production of **fibroblast growth factor (FGF)**.
4. **Asbestosis** is a disease caused by a family of fibrous silicates commonly found in shipyards, insulation, and roofing industries.
   a. **Clinical features**. Many years after exposure, patients complain of dyspnea, chronic dry cough, recurrent respiratory

NOTE

Silica dust in the lungs is ingested by alveolar macrophages, which become damaged. There is then a release of the macrophages' lysosomal enzymes and production of FGF, resulting in fibrotic silicotic nodules.

infections (especially viral), and weight loss. Respiratory failure can occur many years after exposure has ceased. Patients with asbestos exposure are at increased risk of developing bronchogenic cancer as well as **mesothelioma** (pleural and peritoneal). Smoking causes a multiplicative increase in the risk of developing lung cancer. Patients with asbestosis are also at risk of developing renal and gastrointestinal carcinoma.

b. **Pathology.** Smaller asbestos fibers that reach smaller airways and alveoli are phagocytosed by macrophages after being covered with hemosiderin and glycoprotein (**ferruginous body**).

5. **Berylliosis** is due to heavy exposure to airborne beryllium or its salts. Because of its high tensile strength and resistance to heat and fatigue, beryllium is still used in the electronic, ceramic, aerospace, and nuclear energy industries. Disease due to beryllium probably represents a type IV hypersensitivity reaction, with noncaseating granuloma formation and eventual fibrosis. There is an increased incidence of bronchogenic cancer in patients with berylliosis.

C. **Goodpasture's syndrome** is a necrotizing hemorrhagic interstitial pneumonia that can lead to **hemoptysis** (coughing up blood) and **rapidly progressive glomerulonephritis** (with crescent formation). The disease appears to involve antibody recognition of a common pulmonary and renal basement membrane antigen.

1. **Clinical features**. Goodpasture's syndrome usually occurs in young individuals (20's and 30's) and is more common in men. Death usually occurs as a result of complications of renal failure, but massive hemoptysis can be responsible.

2. **Pathology**

a. **Grossly**, heavy lungs with areas of red-brown consolidation are seen.

IN A NUTSHELL

Goodpasture's syndrome:

Antibodies against glomerular and pulmonary basement membranes result in a hemorrhagic pneumonitis and glomerulonephritis. Immunofluorescence reveals linear deposits of IgG along the glomerular basement membrane. If you see a patient with both hemoptysis and hematuria, think Goodpasture's.

VASCULAR DISORDERS

A. **Pulmonary congestion and edema** result from an accumulation of fluid and protein within the pulmonary interstitium and alveolar space as a result of hemodynamic (Starling's) derangements or from increased capillary/alveolar permeability.

1. Most commonly, **pulmonary edema** develops when there is an increase in pulmonary capillary pressure as with left heart failure. Volume overload of the nephrotic syndrome and decreased lymphatic drainage also lead to transudation of fluid across the alveolar membrane. As fluid accumulates in the interstitium, interendothelial junctions stretch, leading to increased permeability to both fluid and macromolecules. The lymphatic flow must be increased 10-fold before the lung's drainage mechanism is overwhelmed, leading to edema. It is only after even higher capillary pressures are achieved that fluid moves from the interstitium into the alveolar space.
2. **Alveolocapillary permeability.** Edema results after injury to both capillary endothelial and alveolar epithelial cells. Fluid and protein accumulate initially in the interstitium and subsequently in the alveolar space. **Noncardiogenic pulmonary edema** can result from septic shock, pancreatitis, burns, toxin inhalation, oxygen toxicity, narcotic overdose, pneumonia, organic solvent hypersensitivity, and other causes. Pathologically, the lungs are heavy, wet, and subcrepitant, mostly involving the bases. Alveolar capillaries are engorged, and the alveolar space contains a granular pink precipitate. Alveolar microhemorrhage and hemosiderin-containing macrophages are present (Figure 5-3). If the process becomes

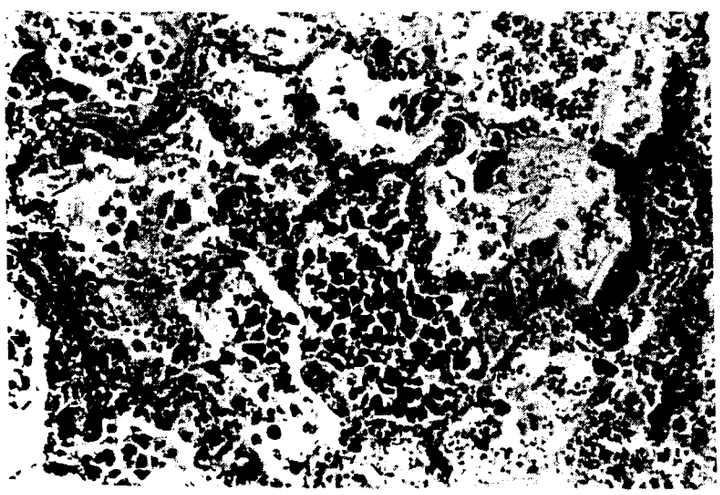


Figure 5-3. Chronic passive congestion with hemosiderin-filled macrophages (microscopic).

IN A NUTSHELL

Cardiogenic pulmonary edema:

- *Left ventricular failure*
- *Mitral stenosis*

Noncardiogenic pulmonary edema:

- *Septic shock*
- *Pancreatitis*
- *Burns*
- *Toxin inhalation*
- *O_2 toxicity*
- *Narcotic overdose*
- *Pneumonia*
- *Organic solvents*

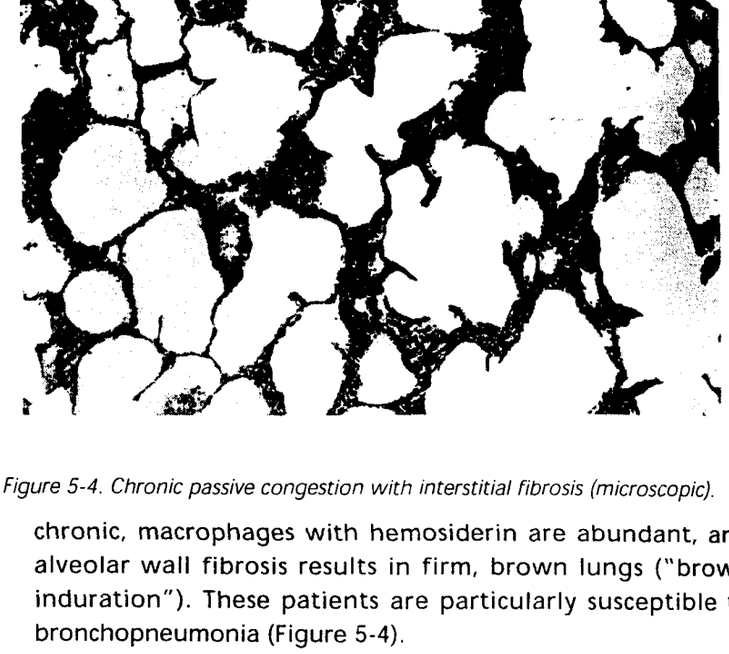


Figure 5-4. Chronic passive congestion with interstitial fibrosis (microscopic).

chronic, macrophages with hemosiderin are abundant, and alveolar wall fibrosis results in firm, brown lungs ("brown induration"). These patients are particularly susceptible to bronchopneumonia (Figure 5-4).

B. **Pulmonary hypertension.** The pulmonary circulation is characterized by low pressure and low resistance, which protect the right ventricle from excessive work. Pulmonary hypertension usually occurs as a result of elevated pulmonary vascular resistance.

1. **Primary pulmonary hypertension** has an unclear etiology, although there are numerous theories. It generally affects young women 20-40 years of age. Some theories include:
   a. **Multiple small pulmonary emboli,** which become organized and incorporated within arterial walls
   b. **Neurohormonal-induced vascular hyperreactivity,** causing chronic vasoconstriction and pulmonary hypertension
   c. **Immune complex-mediated disease**
   d. **Diet or medicinal products,** such as appetite suppressants, which may cause direct endothelial damage
2. **Secondary pulmonary hypertension** results from known diseases, causing elevated pulmonary vascular resistance and pulmonary pressures.
   a. **Increased pulmonary blood flow** may be due to atrial septal defect, ventricular septal defect, patent ductus arteriosis, or Eisenmenger's complex.
   b. **Hypoxic vasoconstriction** may be seen in COPD and interstitial lung disease.

c. **Elevated left heart pressures,** transmitted back to the right side of heart, may occur in congestive heart failure, mitral stenosis, and left atrial myxoma.

d. **Destruction of pulmonary vessels** may occur in schistosomiasis, necrotizing vasculitis, multiple pulmonary emboli, sickle cell anemia, scleroderma, and COPD.

3. **Pathology.** A variety of vascular lesions with much overlap between primary and secondary hypertension is seen.

   a. In **primary hypertension,** medium-sized muscular arteries develop medial hypertrophy, intimal thickening and fibrosis with adventitial fibrosis, and internal and external elastic membrane thickening and reduplication. Small arteries and arterioles are most affected with medial thickening. A "plexiform lesion" may form, consisting of cellular intraluminal angiomatous tufts.

   b. **Secondary changes** are similar to those in the primary disease but may have organized thrombi and diffuse atherosclerotic changes without calcification or ulceration.

4. **Clinical course.** Patients become symptomatic only after the disease is well advanced. They usually present with dyspnea and fatigue. Occasionally, syncope or angina can be the initial manifestation. Respiratory failure or decompensated cor pulmonale result in death within several years of presentation.

C. **Pulmonary thromboembolism and infarction** is an underdiagnosed entity (500,000 annually; 10% fatal), resulting in occlusion of a pulmonary artery by an **embolic blood clot.** Thrombosis on top of a nonocclusive embolus may lead to complete arterial obstruction. The usual sources of emboli are the **deep veins of the leg.** However, a clot can also develop in the pelvic veins and right heart.

1. **Risk factors** include bed-bound conditions, obesity, cancer, pregnancy, oral contraceptives, hypercoagulability, and prior deep venous thrombosis.

   a. **Large emboli** may occlude the main pulmonary artery or its major branches or lodge in the pulmonary artery bifurcation, leading to a "saddle embolus." Sudden death can follow from blockage of blood flow out of the right ventricle or from acute right heart failure (acute cor pulmonale).

   b. **Small emboli** occlude smaller vessels. Fewer than 10% of pulmonary emboli cause infarction as a result of bronchial artery collateral flow to the lung parenchyma. Under these conditions, hemorrhage with parenchymal preservation rather than infarction occurs. If the collateral circulation is compromised, even small emboli can cause infarction.

IN A NUTSHELL

Pulmonary embolism:

- *Very common occurence*
- *Occurs during times of venous stasis, especially during prolonged bed rest or sitting, CHF, and in primary venous disease*
- *Most often originates from a "DVT" or deep venous thrombosis in the lower extremities or pelvic area*
- *Risk factors include: obesity, cancer, pregnancy, oral contraceptives, hypercoagulability, multiple fractures and prior DVT*
- *If you are given a question on the exam where a bedridden patient (often post-surgical) develops sudden shortness of breath, think pulmonary embolism. Diagnosis would be confirmed with a $\dot{V}/\dot{Q}$ (ventilation/perfusion) scan.*

Figure 5-5. Pulmonary infarct (gross).

2. **Pathology.** Characteristically, infarctions extend to the lung periphery, forming a wedge-shaped, pleural-based infiltrate. Initially, the infarct is hemorrhagic with ischemic necrosis ("red infarct") (Figure 5-5). Fibrinous exudate forms on the apposed pleural surface. RBCs lyse within 48 hours, and eventually fibrous replacement begins at the margins, leading to scar formation.

3. **Clinical features** of a pulmonary embolus depend on its size.
   a. **Small emboli** cause transient cough, dyspnea, tachycardia, hyperventilation, and possibly chest pain. Infarction may produce fever, worsening chest pain, and hemoptysis in addition to dyspnea and tachypnea.
   b. **Large emboli** can produce sudden death with a clinical syndrome similar to an acute myocardial infarction (chest pain, severe dyspnea, shock, fever).

D. **Fat embolism** is characterized by progressive respiratory insufficiency, mental deterioration, and occasionally renal insufficiency. These emboli usually develop 1-3 days after a **long bone fracture.**

1. **Pathogenesis** is controversial and probably multifactorial.
   a. Release of fat globules from the marrow may simply occlude vessels in the lung and brain. Smaller globules may fit through the pulmonary vasculature and cause systemic emboli.
   b. Chylomicrons may coalesce with stress, leading to vessel occlusion.
   c. DIC may cause obstructive symptoms, exacerbated by fat emboli.

d. Free fatty acids may cause microvascular toxic injury, leading to capillary block.

2. **Prognosis.** Mortality is high (10%–15%).

E. **Amniotic fluid embolism.** Release of thrombogenic amniotic fluid into the maternal circulation during delivery causes widespread thrombosis and occlusion of pulmonary capillaries. DIC may follow. There is a high mortality rate.

LUNG TUMORS

Most lung tumors represent metastatic lesions. Of the primary lung neoplasms, most are **bronchogenic carcinomas.**

A. **Benign neoplasms**

1. **Hamartomas** are the most common benign pulmonary neoplasm. They are **mesenchymal neoplasms,** composed of a mixture of tissues usually found in the lung (cartilage, smooth muscle, collagen) in a disorganized array. They can become extremely large despite their benign nature and can remain clinically silent because of their peripheral location. Calcification resembling "popped popcorn" occurs in 5%–20% of hamartomas.
2. **Bronchial adenomas** arise from bronchial mucous glands.
3. **Leiomyomas** arise from smooth muscle, usually in an endobronchial location.
4. **Hemangiomas** are usually peripheral and often subpleural.
5. **Lipomas** are usually endobronchial and can occur on either side of the bronchial cartilage.
6. **Chondromas** are derived exclusively from formed bronchial cartilage.

B. **Bronchial carcinoids** comprise up to 5% of all primary lung tumors. They are a disease of young adults (35-45 years of age). Smoking does not appear to be an independent risk factor. The cells are derived from a precursor cell, closely related to the Kulchitsky **neuroendocrine argentaffin cell** and contain neurosecretory granules. The release of neuroendocrine substances leads to the carcinoid syndrome.

1. **Clinical features**. Eighty percent of bronchial carcinoids are central lesions that are "radiographically silent" but can lead to bronchial obstruction, causing cough, fever, chest pain, and localized wheeze. Hemoptysis is present in approximately 50%, reflecting central origin and hypervascularity. Complete obstruction can lead to bronchiectasis and parenchymal necro-

sis distal to the obstruction. Twenty percent are peripheral lesions that are usually clinically silent; they are detected fortuitously on routine chest x-ray as a slightly lobulated nodule. Calcification is rare. Only 3.5% develop the carcinoid syndrome with diarrhea, cutaneous flushing, wheezing, heart disease (valvular fibrosis), abdominal pain, and telangiectasia.

C. **Bronchogenic carcinoma** is the leading cause of cancer death among both men and women. The female preponderance has increased, most probably as a result of increased smoking among women in the past few decades. Bronchogenic carcinoma occurs most commonly in patients 40–70 years of age. **Adenocarcinoma** is the most frequent type of bronchogenic carcinoma, surpassing squamous cell carcinoma. Carcinoma of the lung begins as an area of cellular hyperplasia and atypia that causes thickening of the bronchial mucosa. Eventually, an irregular elevation forms that can elevate or erode the lining epithelium. Continued progression can follow one of three paths: **intraluminal growth, infiltrative peribronchial growth, and intraparenchymal cauliflower-like growth** that pushes normal tissue away. When bulky, hemorrhage or necrosis can convert the usual grey-white firm mass to a yellow-white mottled and softer mass. Spread to hilar, mediastinal, bronchial, and tracheal lymph nodes is common (50%). Metastasis via lymphatics or blood occurs relatively early. Only approximately 25% of lung cancers are operable when discovered.

1. **Types**
   a. **Adenocarcinoma** (35%) usually forms peripheral tumors that arise from distal airways and alveoli, although occasionally they occur proximally, arising from submucosal glands or epithelium. Adenocarcinoma occurs equally in men and women, and is less closely associated with smoking than squamous cell.
   b. **Squamous cell** (25%) arises from bronchial epithelium, following years of mucosal alterations, including metaplasia, dysplasia, and carcinoma *in situ*. The tumor starts as a small red granular plaque or as a focus of whitish leukoplakia, and progresses to a large intrabronchial mass. Cavitation may occur in the lung distal to the mass. Squamous cell carcinoma is most closely related to **cigarette smoking**. It tends to metastasize locally and somewhat later than the other lung tumors. It is more common in men and is usually centrally located.
   c. **Small cell carcinoma** (25%), forms proximal, large, soft, grey-white masses that can narrow bronchi circumferentially simply by extraluminal tumor bulk. There is rapid growth

NOTE

Small cell carcinoma cells secrete the hormones ACTH and ADH. This may give rise to a Cushing's syndrome or syndrome of inappropriate ADH (SIADH), respectively. Squamous cell carcinoma may secrete a parathyroid hormone-like substance that may cause hypercalcemia.

and early dissemination so that, if untreated, the median survival is less than 3 months.

d. **Large cell carcinoma** (15%) forms peripheral, anaplastic lesions that can become quite large and active.

e. **Bronchioalveolar carcinoma** (5%) is a subset of adenocarcinoma that arises from terminal bronchioles or alveolar walls.

2. **Major risk factors**

a. **Cigarette smoking.** The incidence of lung cancer is related to the number of cigarettes smoked per day, the duration of cigarette use, the depth of inhalation, and the type of cigarette used. Histologic changes in the bronchial epithelium caused by smoking include:
(1) Loss of bronchial cilia
(2) Basal epithelial hyperplasia
(3) Nuclear hyperchromatism

b. **Occupational exposure,** including uranium mining, metal work, painting, and radiation, all may increase the risk of cancer.

c. **Air pollution.** Reducing agents (sulfur dioxide and carbonaceous particulate matter) appear to be carcinogenic, whereas oxidants are not.

d. **Genetics.** There may be a familial predisposition to lung cancer, particularly with deletions or mutations to p53 or the retinoblastoma gene.

3. **Clinical features**. There are two modes: early and late, depending upon cell type and site of origin. Staging of disease is by the size of the tumor, number of affected nodes, and distant metastasis (TNM system). In the early stage of disease, intrabronchial lesions cause mild cough or a change in the character of a chronic cough. Partial obstruction may produce focal emphysema. Total occlusion leads to postobstructive atelectasis or pneumonia with fever, chills, sputum production, localized wheeze, hemoptysis, or abscess formation. In the late mode, there is a wide spectrum of presentations.

a. Nonspecific systemic symptoms include weight loss, anorexia, fatigue, weakness, and nausea.

b. Intrathoracic spread can lead to Horner's syndrome with secondary cervical sympathetic nerve involvement, **superior vena cava syndrome**, dysphagia with secondary esophageal obstruction, hoarseness with secondary recurrent laryngeal nerve involvement, diaphragmatic paralysis with secondary phrenic nerve damage, and **Pancoast tumor** (causing ulnar nerve pain and Horner's syndrome).

NOTE

Superior vena cava (SVC) syndrome may be a presentation of bronchogenic carcinoma. In this syndrome, obstruction of the SVC by tumor results in dilatation of head and neck veins, facial swelling, and cyanosis.

CLINICAL CORRELATE

Sputum cytology, transbronchial biopsies, and open biopsies are all used to diagnose lung cancer. Pathologists are also asked to evaluate resection margins following surgery as well as to detect the presence of lymph node metastases. Evaluation of liver and bone marrow biopsies are required in the setting of metastatic disease for the purpose of staging.

c. Extrathoracic extension may involve prescalene lymph nodes, brain, liver, adrenal, and, most commonly, **bone metastases.**

d. The systemic syndromes, or **paraneoplastic syndromes,** may occur before the lesion is visible on x-ray.

(1) Endocrine/metabolic syndromes are listed in Table 5-1.

(2) **Neuromuscular syndromes** include cerebral encephalopathy and cortical cerebellar degeneration (small cell), peripheral neuropathy with pain, paresthesias, myasthenia (Eaton-Lambert syndrome), and proximal muscle neuromyopathy.

(3) **Hematologic/vascular syndromes** include anemia unrelated to therapy or bone marrow infiltration, coagulopathy (Trousseau's syndrome), migratory thrombophlebitis, DIC, noninfectious endocarditis, and arterial embolization.

(4) **Dermatologic signs** are dermatomyositis, hyperpigmentation, and acanthosis nigricans.

(5) **Skeletal and connective tissue syndromes** include hypertrophic pulmonary osteoarthropathy (periosteal new bone formation, clubbing, and arthritis), which is sometimes caused by squamous cell carcinoma. Vasomotor instability with blanching of hands and feet may also be seen.

Hormone secreted by tumor	Pathophysiologic consequences
ACTH	Cushing's syndrome (rare)
MSH	Increased skin pigmentation
PTH	Hypercalcemia, often due to squamous cell carcinoma
ADH	Hyponatremia
HCG	Gynecomastia (large cell)
Prolactin	Lactation in men or women
VIP	Diarrhea, hypokalemia, achlorhydria (squamous cell)
Calcitonin	Hypocalcemia

ACTH = adrenocorticotropic hormone; MSH = melanocyte-stimulating hormone; PTH = parathyroid hormone; ADH = antidiuretic hormone; HCG = human chorionic gonadotropin; and VIP = vasoactive intestinal polypeptide.

Table 5-1. Paraneoplastic syndromes.

MEDIASTINAL MASSES

Mediastinal masses often present as an unexpected finding on routine chest x-ray. Symptoms are due to either compression or invasion of neighboring structures. Vascular lesions may present as "masses" in all parts of the mediastinum and include congenital vascular rings; double superior venae cavae; aortic malformations, aneurysms, and dilatations; aneurysm or dilatation of major aortic branches; and dilated pulmonary arteries. Other masses include diaphragmatic herniations and pulmonary lobar sequestrations. The mediastinum can be divided into three compartments, each with characteristic lesions:

A. **The anterior mediastinum** ranges from the root of the neck, extending down to include the region between the sternum (anteriorly) and pericardial surface (posteriorly).

1. **Thymoma** is the most common anterior mediastinal mass. There are four cell types: epithelial, lymphocytic, spindle, and mixed. Benign thymomas have a thick fibrous capsule and do not invade. Malignant thymomas lack a capsule and do invade.
2. **Teratomas** are tumors derived from pluripotential precursor cells.
   a. **Mature teratomas (dermoid cysts)** generally show ectodermal differentiation, although elements from other germ layers may be present. They are generally benign, although approximately 1% undergo malignant transformation.
   b. **Immature teratomas** have a fetal or embryonic appearance microscopically; primitive neuroepithelial cells are frequently encountered. Immature teratomas may behave aggressively; tumor behavior correlates with histologic grade.
3. **Lymphoma.** The most common lymphoma is nodular sclerosing Hodgkin's disease. Tracheal compression occurs in 20%.
4. **Cysts** of pericardial, bronchogenic, or thymic origin are also rarely seen.
5. **Intrathoracic goiter** is an unusual finding.

B. **The middle mediastinum** includes the pericardium and its contents, lower trachea, carina and main bronchi, and lymph nodes.

1. **Cysts**
   a. **Pericardial cysts** are usually located in the cardiophrenic angle. They occasionally communicate with the pericardial space and are composed of one mesothelial layer, covering a thin fibrous wall.

BRIDGE TO CARDIOVASCULAR

The anatomy of the mediastinum is reviewed in Cardiovascular Anatomy.

BRIDGE TO HEME/LYMPH

Lymphoma is discussed in detail in the Heme/Lymph Pathology chapter.

b. **Bronchogenic cysts** are lined with ciliated columnar epithelium with mucous glands and cartilage in the wall.
c. **Enteric cysts** are lined by squamous epithelium and smooth muscle without cartilage.

2. **Lymphoma,** both Hodgkin's and nonHodgkin's types, may involve middle mediastinal nodes.
3. **Primary mediastinal carcinoma** may arise from cyst epithelium.
4. **Granulomatous lesions.** Histoplasmosis, sarcoidosis, and TB all may involve middle mediastinal nodes, usually because they drain primary lesions in the lungs.

C. **The posterior mediastinum** includes the posterior pericardium to the anterior vertebral column and posterior ribs, including the paravertebral gutters.

1. **Neurogenic tumors** are almost always benign in adults, although 10% have an intraspinal component.
   a. **Schwannomas** (neurilemomas) are benign nerve sheath tumors of Schwann cells.
   b. **Neurofibromas** are benign nerve sheath tumors of fibroblasts.
   c. **Ganglioneuromas** are benign nerve cell tumors of sympathetic ganglion cells. They occur primarily in the second and third decades.
   d. **Ganglioneuroblastomas** are malignant tumors of sympathetic neurons; they are common in children and infants.
   e. **Neuroblastoma** is also common in children and infants and is highly malignant.

DISEASES OF THE PLEURA

A. **Effusions** are abnormal **accumulations of fluid within the pleural space;** they are a common manifestation of both systemic and intrathoracic disease. The normal pleural space contains no more than 15 cc of serous fluid that lubricates the pleural surface. The factors that determine whether pleural fluid accumulates include oncotic pressure in the pleural microcirculation and surrounding tissue, permeability of the pleural microcirculation, pressure in the pleural microcirculation and surrounding tissue, intrathoracic negative pressure, and lymphatic drainage. Pleural effusions can be divided into **transudates** (low lactate dehydrogenase, low protein) and **exudates** (high lactate dehydrogenase; high protein).

1. **Noninflammatory pleural effusions (transudates)**
   a. **Hydrothorax. Noninflammatory serous fluid** collects in the pleural cavity as a result of CHF (increased pressure), renal failure (fluid overload, increased pressure), cirrhosis (fluid overload, decreased oncotic pressure), or nephrotic syndrome (fluid overload, decreased oncotic pressure). The fluid is clear and straw-colored.
   b. **Hemothorax** follows hemorrhage into the pleural space, often the result of a rupturing aortic aneurysm of iatrogenic causes, such as biopsies.
2. **Inflammatory pleural effusions (exudates)**
   a. **Serofibrinous pleuritis** is caused by **inflammatory diseases** within the lung such as TB, pneumonia, lung infarcts, lung abscess, and bronchiectasis. Systemic disease, such as rheumatoid arthritis, systemic lupus erythematosus, uremia, and diffuse infections can also cause serous or serofibrinous pleuritis. The fluid consists of relatively clear, straw-colored fluid with small strands of yellow fibrin and few WBCs.
   b. **Suppurative pleuritis (empyema)** is a **purulent exudate** with bacterial or fungal seeding of the pleural space, usually by contiguous spread from the lung. Occasionally, infection can come from blood or lymphatics. It is characterized by **yellow-green pus** with masses of polys and other leukocytes. Empyema infrequently resolves but usually organizes with the formation of tough fibrous adhesions that can obliterate the pleural space or form a pleural "peel", preventing pulmonary expansion. Calcification is typical of tuberculous empyema.

B. **Pneumothorax** is an accumulation of **air or gas in the pleural cavity**, leading to **collapse of the underlying lung** as a result of increased surrounding pressure (pleural pressure is usually negative). Pneumothorax is frequently due to spontaneous rupture of an alveolus or bleb or to a communication between an abscess and either the pleural space or interstitium. It is most common in patients with emphysema, asthma, and TB. **Traumatic pneumothorax** results from puncture of the chest wall with communication between the pleural space and external environment. When air can enter the pleural space but not exit during expiration, pressure builds, leading to a **tension pneumothorax** with **tracheal deviation,** respiratory compromise, and hemodynamic instability.

C. **Tumors**
   1. **Metastatic involvement** of the pleura is most common, usually from the breast or lung.

BRIDGE TO CARDIOVASCULAR

Now may be a good time to review the Starling equation in the Cardiovascular Physiology section. Transudates result from ↑ P_c, ↓ π_c, or a combination of the two.

IN A NUTSHELL

Transudate	***Exudate***
• *Specific gravity less than 1.012*	• *Specific gravity greater than 1.020*
• *Noninflammatory edema fluid resulting from changes in hydrostatic or osmotic pressure intravascularly*	• *Inflammatory edema fluid with increased vascular permeability*
• *↓ protein in fluid*	• *↑ protein in fluid*
	↓ glucose in fluid
	↑ inflammatory cells in fluid

2. **Malignant mesothelioma** is a rare tumor that arises from parietal or visceral pleura. It is associated with **asbestos exposure** after a prolonged latent period of 25–45 years. In contrast to bronchogenic carcinoma, in which smoking and asbestos exposure act synergistically, smoking does not increase the risk of malignant mesothelioma. The malignant mesothelioma is a diffuse lesion that spreads over the lung surface, causing a pleural effusion and invasion of thoracic structures. The lung is encased by a thick layer of gray-pink tumor, composed of mesenchymal stromal cells or even papillary, epithelial-like cells. Patients complain of chest pain and dyspnea. Prognosis is poor.

LARYNGEAL DISEASES

A. **Inflammation. Laryngitis** is usually part of an inflammatory process of the lung and lower respiratory tract. It may also be involved with diffuse infections, such as TB, syphilis, diphtheria, and local disease of the mouth and throat. Although trivial in the adult, laryngeal inflammation can lead to upper airway obstruction in children.

B. **Tumors**

1. **Benign neoplasms**
   a. **Polyps** usually occur on the true vocal cords as smooth, round nodules that may be pedunculated or sessile. Polyps are composed of loose connective tissue and covered by squamous epithelium that can ulcerate when traumatized by the opposite vocal cord. They are associated with heavy smoking and vocal cord overuse.
   b. **Papilloma** is a true neoplasm, usually a soft, friable nodule on the true vocal cords. Papillomas frequently ulcerate and bleed with manipulation. They are composed of multiple finger-like projections composed of fibrous tissue covered with squamous epithelium. Papillomas rarely undergo malignant transformation.

2. **Malignant tumors** are uncommon except for those arising from the surface epithelium. Most occur on the vocal cords, although they can occur anywhere. Ninety-five percent are **squamous cell carcinomas,** which can cause hoarseness, difficulty swallowing, pain, hemoptysis, and eventually, respiratory compromise. Ulceration can lead to superinfection. Complications arise due to direct extension, metastases, and infection. Risk factors include cigarette smoking, alcohol, and frequent cord irritation.

Nervous System Pathology

The nervous system is affected by all of the same processes that affect other organ systems: genetic malformations, degeneration, infectious diseases, vascular insufficiency, and neoplasms.

Degenerative disorders of the central nervous system (CNS) are often due to the accumulation of metabolic products, sometimes from an inherited disorder (e.g., Tay-Sachs disease) and sometimes from a problem acquired or manifested later in life (e.g., Alzheimer's disease). A great deal of progress has been made in determining the molecular basis of these diseases. Prenatal tests now exist for Tay-Sachs disease, Huntington's disease, and even for a genotype predisposing to Alzheimer's disease.

Vascular disease is of primary importance in the CNS. Again, arteriosclerosis and its attendant risk factors are the most common cause of vascular insufficiency and its resultant pathology, stroke. Infectious diseases of the CNS can have viral, bacterial, protozoal, or fungal etiologies. Some of the "slow virus" diseases such as kuru have now been shown to be due to prions, which are thought to be proteins that may fold into more than one configuration.

NONSPECIFIC NEURONAL AND GLIAL CHANGES

The following terms describe conditions that may be seen alone or in combination in many disorders that affect the central nervous system (CNS). They are presented here together for convenience, but will be discussed again throughout the chapter.

A. **Neuronal loss** is the endpoint of many disease processes. It usually requires at least a 30% loss of neurons before it is observable by

light microscopy. In many cases, neuronal loss is accompanied by fibrous gliosis.

B. **Ischemic neuronal damage** is an acute process that most commonly follows anoxia. It is characterized by retraction of the cell body (soma), disappearance of Nissl substance, cytoplasmic eosinophilia, and nuclear pyknosis (condensation, often with hyperchromasia).

C. **Neurophagia** refers to neuronal phagocytosis, which often occurs with viral infections; the degenerating neuron is surrounded by monocytes and microglia (CNS macrophages).

D. **Central chromatolysis** (axonal reaction) refers to the reaction of the cell body following a lesion of the lower motor neuron (LMN) axon. The soma swells, Nissl substance disappears (especially around the nucleus), and there is peripheral displacement of the nucleus.

E. **Neuronal atrophy** results from a variety of slowly progressive degenerative processes. The soma shrinks, there is increased cytoplasmic basophilia, nuclear pyknosis, and increased neurofibril and lipofuscin pigment (the "wear and tear" pigment, which accumulates in degenerating and aged neurons and other tissues).

F. **Gliosis.** In gliosis, injury to the CNS stimulates hypertrophy and hyperplasia of astrocytes shortly after exposure to a variety of noxious agents. The cell body, nucleus, and processes of the astrocyte swell. In the chronic stage, glial fibers accumulate as the cell body shrinks. Roughly 5 days after infarction, swollen astrocytes with eosinophilic cytoplasm proliferate around the necrotic lesion.

IN A NUTSHELL

Signs of ischemic neuronal damage:

- *Nissl substance dissolution*
- *Cytoplasmic eosinophilia*
- *Nuclear condensation (pyknosis)*

IN A NUTSHELL

Gliosis is the scarring process of the CNS. Glial scars are formed by astrocytes (and sometimes by fibroblasts).

DEGENERATIVE DISORDERS OF THE CNS

Degenerative disorders of the CNS are a mixed group of disorders that tend to begin insidiously and progress gradually. They may cause dementia, disorders of movement and posture, ataxia, weakness, or sensory changes.

A. **Alzheimer's disease** is the most common form of dementia. Presenile and senile forms have been distinguished on the basis of age, although both share common clinical and pathologic features and are considered together.

1. **Incidence.** Most cases occur after the age of 40. There is a female predominance.
2. **Etiology** remains unknown, although it has been ascertained that genetic factors are involved in a small number of cases.

3. **Clinical features.** Early symptoms include impairment of short-term memory, abstract thinking, problem solving, and visuospatial orientation, as well as emotional and social changes (e.g., irritability). Symptoms progress and are later accompanied by aphasia and apraxia; ultimately, the patient enters a vegetative state.
4. **Pathology**
   a. **Grossly,** diffuse **cortical atrophy** occurs with relative sparing of primary motor and sensory areas. Gyri are thin and sulci are wide.
   b. **Microscopically**, neuronal loss occurs mainly in the cortex but also in many subcortical nuclei. The following features are characteristic, though not pathognomonic.
      (1) **Neurofibrillary tangles** are intracytoplasmic, skein-like structures composed of paired helical filaments.
      (2) **Granulovacuolar degeneration** describes small cytoplasmic vacuoles containing a central granule.
      (3) **Senile plaques** are abnormal, enlarged, presynaptic axon terminals surrounding a central core of extracellular amyloid-like substance.
      (4) **Hirano bodies** are found in some cases.
   c. It has been proposed that loss of cholinergic neurons in the nucleus basalis of Meynert is in part responsible for memory impairment and other cognitive deficits.

IN A NUTSHELL

Alzheimer's pathology:

- *Gross cortical atrophy*
- *Microscopic changes*
  - *Neurofibrillary tangles*
  - *Senile plaques*
  - *Hirano bodies*

B. **Pick's disease** is a rare form of dementia, which causes lobar atrophy (affecting both grey and white matter), predominantly in the frontal and temporal lobes. Familial cases are common.

IN A NUTSHELL

Pick's disease is similar to Alzheimer's disease, but with atrophy localized to the frontal and temporal lobes.

C. **Parkinson's disease** (paralysis agitans) is an idiopathic disorder that usually begins after age 40 and afflicts 1% of the population older than 50.

1. **Clinical features** include bradykinesia (difficulty initiating and slowness of voluntary movement), rigidity, resting tremor, flexed posture, expressionless (masked) facies, and festinating (shuffling) gait. Dementia may occur.
2. **Etiology.** Symptoms are primarily from **dopamine depletion in the caudate and putamen,** the termination of the nigrostriatal tract. The cause of death of substantia nigra dopaminergic neurons is unknown.
3. **Pathology**
   a. **Grossly, depigmentation of the substantia nigra** (which contains the somata of dopaminergic neurons from which the nigrostriatal tract originates) and locus ceruleus is evident.
4. **Parkinsonism** includes disorders displaying the clinical features of Parkinson's disease. This classification is now recognized as encompassing several routes of pathogenesis. The final com-

IN A NUTSHELL

Parkinson's symptoms:

- *Bradykinesia*
- *Rigidity*
- *Resting tremor*
- *Festinating gait*
- *Masked facies*
- *Dementia*

Parkinson's disease is characterized by a loss of dopaminergic neurons in the substantia nigra, which projects to the striatum (caudate nucleus and putamen).

mon pathway for the recognized etiologies listed below is thought to be nigrostriatal dopamine depletion or blockade of postsynaptic receptors. Causes include:

a. Neuroleptics (e.g., phenothiazines)
b. Encephalitis, particularly viral
c. Carbon monoxide/manganese poisoning
d. Strokes
e. Methylphenyltetrahydropyridine (MPTP), which is found in some synthetic heroin

D. **Huntington's disease** is characterized by **autosomal dominant inheritance, choreoathetosis,** and **dementia.**

1. **Incidence.** The onset is usually between 25 and 55 years of age and tends to be about the same in each afflicted family.
2. **Pathology**
   a. **Grossly**, there is striking degeneration of the medium-sized, spiny neurons of the **caudate nucleus** with less severe involvement of the putamen and cerebral cortex.

IN A NUTSHELL

Huntington's disease is an autosomally dominant disease characterized by the degeneration of neurons of the caudate nucleus. The gene is localized to the short arm of chromosome 4.

E. **Progressive supranuclear palsy** is a degenerative disorder characterized by ophthalmoplegia (affecting vertical before horizontal gaze), pseudobulbar palsy, axial dystonia, and bradykinesia. Mild dementia often develops.

1. **Incidence.** Onset is usually between the fifth and seventh decades of life.
2. **Pathology**
   a. **Grossly,** there is widespread neuronal loss and gliosis in subcortical sites with sparing of the cerebral and cerebellar cortices.

F. **Friedreich's ataxia** is an inherited disorder (usually autosomal recessive).

1. **Incidence.** Onset is usually between ages 5 and 25.
2. **Clinical features.** Most patients are unable to walk within 5-10 years of onset because of progressive ataxia. Associated features include pes cavus (hollowing of the instep), diabetes, kyphoscoliosis, diminished proprioception, tremors, decreased or absent tendon reflexes, Babinski's sign, and cardiomyopathy.

G. **Motor system disease** refers to a group of overlapping degenerative disorders characterized by some combination of **muscle weakness, atrophy,** and **spasticity;** these symptoms result from loss of motor cells in the spinal cord, brain stem, cerebral cortex, and cerebellum. These disorders include **amyotrophic lateral sclerosis** (ALS; Lou Gehrig's disease).

CLINICAL CORRELATE

In ALS, both upper and lower motor neurons degenerate. Symptoms are mixed: atrophy and fasciculation indicate lower motor neuron degeneration; spastic paralysis, increased muscle tone, and hyperreflexia indicate upper motor neuron degeneration.

VASCULAR LESIONS

A. **Cerebral infarction** refers to necrosis of neural parenchyma secondary to inadequate blood or oxygen supply.

1. **Etiology**
   a. **Thrombosis** usually results from atherosclerosis. Diabetes, smoking, family history, age, hypertension, and alcohol are important risk factors. Thrombosis is also caused by arteritis, vascular trauma, and a hypercoagulable state. It usually occurs in large- and medium-sized vessels.
   b. **Emboli** may arise from a mural thrombus of the left ventricle, aortic or carotid plaques, septic emboli, and fat and air emboli. They usually occur in medium-sized vessels.
   c. **Lacunar infarcts** are clues to the occlusion of deep penetrating arteries and are associated with hypertension. They are named for small cavities (lacunes) formed in the deep white or grey matter.
2. **Clinical features** vary with etiology and location.
   a. **Thrombosis** follows a variable course, often stuttering and often preceded by **transient ischemic attacks (TIAs),** which cause focal neurologic dysfunction lasting up to 24 hours.
   b. **Emboli** often produce their maximal deficit within 1 minute; signs and symptoms depend on the specific arterial territory affected.

B. **Intracranial hemorrhage.** Bleeding within the cranial cavity may occur in the epidural, subdural, or subarachnoid spaces, or in neural parenchyma. Epidural and subdural hemorrhages are discussed under trauma (Figure 6-1).

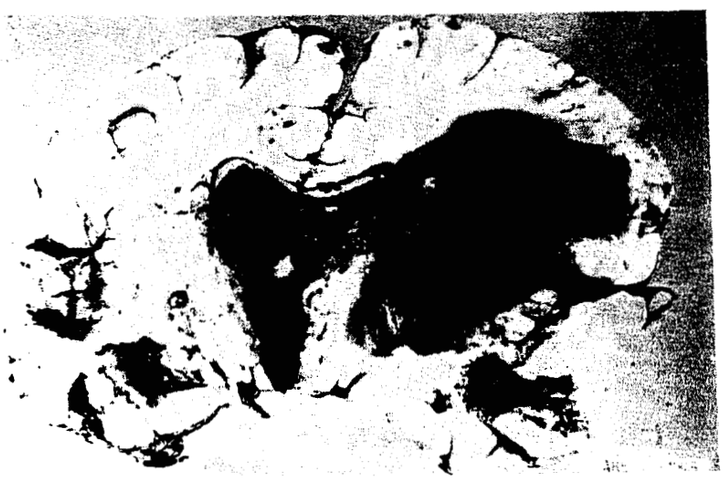


Figure 6-1. Massive right intracerebral hemorrhage (gross).

IN A NUTSHELL

Thrombotic infarcts, characterized by permanent neural damage, are often preceded by TIAs, which are temporary syndromes resembling mini-strokes.

IN A NUTSHELL

Intraparenchymal bleeds, often caused by hypertension, are the most common cause of stroke fatality.

1. **Intraparenchymal bleeds** are usually the result of **hypertension** (called hypertensive, primary, or spontaneous intracerebral hemorrhage) and are the most common cause of death from stroke.
   a. **Clinical features**
      (1) There is a sudden headache and abrupt onset of neurologic deficit.
      (2) Edema may be massive, and herniation can occur.
      (3) The CSF is usually bloody, especially in hypertensive hemorrhage with dissection of blood into the ventricular system.

IN A NUTSHELL

Ruptured berry aneurysms are the most frequent cause of subarachnoid hemorrhage; they often occur at bifurcations of the anterior circle of Willis.

2. **Saccular (berry) aneurysms** are the most common cause of nontraumatic subarachnoid hemorrhage.
   a. **Etiology** is usually attributed to congenital defects in the arterial media, acquired factors such as atherosclerosis and hypertension, or both.
   b. **Pathology.**
      (1) Ninety percent of saccular aneurysms are in the anterior part of the **circle of Willis,** especially at bifurcations. In order of frequency, sites of rupture are the posterior and anterior communicating arteries and the bifurcation of the middle cerebral artery.
   c. **Clinical features**. Rupture usually causes **severe headache,** which may be followed by no deficit or may be followed by coma. Rupture often occurs during exertion but may occur spontaneously.

CLINICAL CORRELATE

Patients with subarachnoid hemorrhages often describe having the "the worst headache of my life."

C. **Arteriovenous malformations (AVMs)** are developmental abnormalities (non-neoplastic) that directly connect arterial and venous circulations without capillaries. These fistulae vary in size and may be found throughout the CNS. Ninety percent are found in the cerebral hemispheres. There is a male predominance.

BRAIN TRAUMA

IN A NUTSHELL

- *Concussion → no structural damage*
- *Contusion → "brain bruise" from blunt head trauma*

A. **Concussion** is a transient paralysis of cerebral function immediately after head trauma (typically a blunt, nonpenetrating injury such as a blow with a fist) that is not associated with structural damage. Although impairment of consciousness is brief, symptoms (e.g., headache, dizziness) may persist. Duration of posttraumatic amnesia is the best index of the severity of injury.

B. **Contusion** is a bruise of the brain parenchyma that typically involves the summit of the gyrus. The bruise produces a wedge-shaped defect of necrosis and petechial hemorrhages with the base near the meninges and the apex towards the white matter.

C. **Skull fractures** may be of no clinical significance, or they may be responsible for important sequelae, including contusion (usually with depressed fractures), CSF leakage (meningeal tear), or epidural hematoma (vascular tear).

1. **Linear fractures** are seen as lucent lines with well-defined borders. Both tables of the skull are involved.
2. **Depressed fractures** cause indentation of the skull and are often associated with contusion.
3. **Compound fractures** have a communication with the outside through an associated tear of the scalp or paranasal sinuses. Osteomyelitis may develop.
4. **Basilar skull fractures** are usually linear and may not be seen on x-ray. CSF leak and cranial nerve palsies may develop.

D. **Hemorrhage** following head trauma may occur into the epidural, subdural, or subarachnoid spaces or within the parenchyma of the brain.

1. **Epidural** (extradural) hemorrhage occurs into the space between the dura and the skull; bleeding may arise from arteries, veins, or both. Most cases follow trauma to the lateral skull, resulting in **laceration of the middle meningeal artery,** although frontal and occipital lesions also occur. Skull fracture is present in 80%-90% of cases. Classically, the head trauma is associated with momentary loss of consciousness, followed by a lucid (asymptomatic) period of 1-48 hours. The patient then develops symptoms of elevated intracranial pressure (e.g., headache, changes in mental status, nausea, vomiting) and possibly focal findings (e.g., hemiparesis). Herniation of the medial temporal lobe, coma, and death may result if the collection of blood is not surgically evacuated.
2. **Subdural hematoma** results from bleeding into the space between the dura and arachnoid.
   a. **Acute subdural hematomas** almost always result from severe head trauma, causing **tears in the bridging veins;** they are associated with contusion. Large hematomas are usually fatal; smaller ones may lead to symptoms after a latent interval of days to weeks. Treatment consists of surgical evacuation of the clot.
   b. **Chronic subdural hematoma.** The diagnosis of a chronic subdural hematoma is often difficult because many patients are elderly or alcoholic, and head trauma may be minor or forgotten. Anticoagulation and coagulopathy are predisposing factors. Symptoms may develop weeks to months after trauma. Headache, drowsiness, asymmetric

IN A NUTSHELL

- *Epidural hematomas lie between the skull and the dura. Due to their arterial origin, they grow rapidly and constitute a serious emergency.*
- *Subdural hematomas lie between the dura and the arachnoid. They originate from the bridging veins and therefore develop more slowly than do epidural hematomas.*

CLINICAL CORRELATE

Because of cortical atrophy due to age, bridging veins are more fragile in the elderly. Thus, elderly persons are more more likely to develop subdural hematomas than are younger persons.

signs, and fluctuation of symptoms are often present. Herniation, coma, and death may result from compressive effects of an enlarging hematoma.

3. **Subarachnoid hemorrhage** results from bleeding into the space between the arachnoid and pia (i.e., subarachnoid space). Severe head trauma or rupture of an aneurysm can produce subarachnoid hemorrhage.
4. **Intracerebral hemorrhage** results from bleeding into the parenchyma of the brain. Although this is an unusual complication of head trauma, it is present in almost half of fatal cases.

E. **Traumatic spinal cord lesions**

1. **Etiology.** Trauma may be penetrating (producing laceration and hemorrhage) or compressive (causing contusion and ischemia). Vertebral bodies may or may not be displaced.
2. **Clinical features** include weakness, parasthesias, and paralysis, depending on the level of the spinal cord involved.
3. **Pathology.** The injured cord undergoes necrosis and hemorrhage and then, ultimately, cavitation and gliosis.

BRAIN EDEMA

Cerebral edema is an important complication of many neurologic diseases. There are three types of brain edema.

A. **Vasogenic edema** results from increased permeability of endothelial cells in brain capillaries. It occurs with trauma, infarction, tumor, infection, hemorrhage, and lead encephalopathy.

B. **Cytotoxic edema** results from the swelling of neurons, glia, and endothelial cells in brain capillaries. It occurs with infarction, hypoxia, or hypo-osmolarity.

C. **Interstitial edema** occurs in obstructive hydrocephalus with leakage of CSF into the periventricular white matter

IN A NUTSHELL

- *Vasogenic edema → interstitium swells*
- *Cytotoxic edema → cells swell*

TUMORS

A. **Overview**

1. **Types.** CNS tumors may be classified as primary or secondary. Secondary tumors include metastatic or craniovertebral bone tumors. Primary tumors may be divided by tissue of origin.
2. **Incidence.** Neoplasm is the second most common cause of mortality from intracranial disease (stroke is first). CNS tumors are found in about 1% of routine autopsies and constitute roughly 9% of all neoplasms.

a. **Neural tube gliomas** include astrocytomas, glioblastomas, ependymomas, oligodendrogliomas, medulloblastomas, ganglioneuromas, and certain pineal tumors.
b. **Neural crest tumors** include meningiomas, schwannomas, and neurofibromas.
c. **Mesodermal tumors** include CNS lymphomas, hemangioblastomas, lipomas, and chordomas.
d. **Ectodermal tumors** include craniopharyngiomas and pituitary adenomas.
e. **Germ cell tumors** include germinomas and teratomas.

3. **Clinical syndromes.** Brain tumors produce progressive cerebral dysfunction. Symptoms may be generalized (if they result from diffuse compromise) or focal. Since the volume of the intracranial cavity is fixed, tumor growth and edema may cause compression or displacement of parenchyma as well as increased intracranial pressure. Obstructive hydrocephalus may result from a block in normal CSF flow. Elevated intracranial pressure may produce headache, nausea, vomiting or papilledema, and autonomic changes (e.g., hypertension, bradycardia, respiratory changes). Pressure gradients in different intracranial compartments can lead to brain herniation. Focal symptoms include aphasia, hemiparesis, and visual field cuts. Generalized or focal seizures occur in roughly 35% of brain tumors. Behavioral changes are common. Stroke-like syndromes may result from hemorrhage into a tumor.

NOTE

Brain tumors produce symptoms directly, by invading tissue, or indirectly, by increasing intracranial pressure.

CLINICAL CORRELATE

Signs and symptoms of increased intracranial pressure:

- *Headache*
- *Nausea and vomiting*
- *Papilledema*
- *Autonomic changes*

NEUROCUTANEOUS DISORDERS

Neurocutaneous disorders or "phakomatoses" are localized tumors and/or tumor-like lesions of the skin, eye, and nervous system.

A. **Neurofibromatosis** has been described above. Neurofibromas rarely become neurofibrosarcomas.
   1. **Clinical features** include:
      a. **Café au lait (pigmented skin lesions) spots**
      b. **Neural tumors** (e.g., neurofibromas)
      c. **Lisch nodules** (benign, pigmented hamartomas of the iris)

B. **Tuberous sclerosis** involves multiple organs but commonly presents as a result of CNS disease, seizures, and mental retardation. Adenoma sebaceum of the face, ashleaf spots, shagreen patches of skin, and subungual angiofibromata may be evident. Multifocal areas of cerebral cortex may be involved with "tubers," which are potato-like masses of giant neurons and astrocytes. The condition is associated with giant-cell astrocytomas, gliomas, and gangliogliomas, and rarely involves the infratentorial CNS or spinal cord.

IN A NUTSHELL

von Hippel-Lindau disease:

- *Autosomal dominant*
- *Hemangioblastomas*
- *Pheochromocytoma*
- *Renal, pancreatic, hepatic cysts*
- *Renal cell carcinoma in up to 50% of patients*

C. **Retinocerebral angiomatosis (von Hippel-Lindau disease)** is an autosomal dominant disorder involving:

1. **Hemangioblastomas** of the retina or cerebellum with other hemangioblastomas in the cerebrum or spinal cord
2. **Abdominal masses,** such as pheochromocytoma
3. **Cysts** of the kidney, pancreas, and liver, and renal cell carcinoma.

D. **Encephalofacial angiomatosis (Sturge-Weber syndrome)** involves the association of an extensive **capillary-venous malformation** of one cerebral hemisphere with an ipsilateral cutaneous **port-wine stain** (nevus flammeus) in the trigeminal region of the face.

MALFORMATIONS AND INTRAUTERINE/PERINATAL LESIONS

IN A NUTSHELL

Failure of the neural tube to close may result in anencephaly, spina bifida, or meningomyelocele. The failure may be associated with maternal folate deficiency, especially during early pregnancy.

A. **Dysraphic states** are malformations that result from incomplete closure in the neural parenchyma or its coverings.

1. **Anencephaly** is a lack of brain formation, resulting from failure of closure of the neural tube at the level of the encephalon. The fetus is always nonviable.
2. **Spina bifida occulta** is dysraphism of the bony spinal canal alone. It is detectable only radiologically.
3. **Meningocele** is a saccular malformation resulting from protrusion of the meninges—but not the spinal cord—through a defect of the bony canal.
4. **Meningomyelocele** is the same as a meningocele except that neural tissue is contained inside the sac. This lesion is frequently seen in association with Arnold-Chiari malformation.
5. **Encephalocele** is a saccular malformation that results from protrusion of the meninges and brain tissue through a defect in the skull.

DEMYELINATING DISEASES

Demyelination may occur primarily (as in multiple sclerosis) or secondary to wallerian degeneration due to axonal death.

A. **Multiple sclerosis (MS)**

1. **Incidence**
   a. MS usually presents between the ages of 20 and 40.
   b. There is a slight female predominance.

2. **Clinical features**
   a. The course is characterized by **spontaneous exacerbations and remissions.**
   b. Ninety percent of patients develop **pyramidal tract dysfunction** (hyperreflexia, weakness, spasticity). Dysfunction is generally multifocal.
   c. Cerebellar dysfunction (e.g., dysarthria, tremor, ataxia) is also common.
   d. Disturbances of extraocular muscles result from lesions of the medial longitudinal fasciculus; disturbances of visual acuity result from lesions of the optic nerve.
3. **Etiology.** There is a presumed autoimmune etiology, possibly influenced by a viral infection. Antibody to measles virus is often detected in the CSF.
4. **Pathology**
   a. **Grossly, plaques are evident in the white matter** (frequently periventricular) or in the corpus callosum. They are also found in the optic nerves and spinal cord.

IN A NUTSHELL

The course of multiple sclerosis is characterized by spontaneous appearance and remission of symptoms.

IN A NUTSHELL

Grossly, MS brains show plaques of demyelination (composed of immune cells and glial cells) in the white matter.

B. **Devic's disease** (neuromyelitis optica) refers to demyelination confined to the optic nerves and spinal cord. It is characterized by blindness, paralysis, and loss of sphincter control.

C. **Postinfectious/postvaccinial encephalomyelitis**
   1. **Pathology.** This form of demyelinating disease causes acute widespread, perivenular demyelination associated with mononuclear infiltration; infiltration follows certain viral illnesses and vaccinations.
   2. **Etiology** is thought to be autoimmune (rather than primary) destruction of myelin by viral infection of the neurons.

D. **Guillain-Barré syndrome** is a demyelinating illness of autoimmune etiology that **affects peripheral nerves** following certain viral illnesses or vaccinations. It usually presents with limb weakness, but facial and ocular muscles may be involved early. Guillain-Barré syndrome may cause complete paralysis, autonomic dysfunction, and respiratory failure. **CSF protein gradually becomes markedly elevated.** Symptoms usually resolve completely, but prolonged respiratory assistance may be required. Peripheral nerves show demyelination and an accumulation of lymphocytes and macrophages.

IN A NUTSHELL

Guillain-Barré syndrome is an autoimmune, postinfectious, peripheral demyelinating disorder. Limb paralysis and autonomic failure may result.

METABOLIC DISORDERS

Metabolic disorders include the sphingolipidoses, mucopolysacch
doses, aminoacidemias, carbohydrate metabolism disorders, Rey
syndrome, Wilson's disease, lupofuscinoses, and deficiency states.

A. **Sphingolipidoses** include many diseases categorized as storag
diseases and leukodystrophies. They are usually categorized by
descriptive term (or eponym), by the enzyme deficiency, or by the
accumulated material.

1. **Specific diseases**
   a. **Niemann-Pick disease** is due to sphingomyelinase deficiency; sphingomyelin accumulates.
   b. **Krabbe's disease** is due to galactocerebrosidase deficiency; galactocerebroside accumulates.
   c. **Metachromatic leukodystrophy** is due to aryl sulfatase deficiency; sulfatide accumulates.
   d. **Gaucher's disease** is due to glucocerebrosidase deficiency; glucocerebroside accumulates.
   e. **Fabry's disease** is due to α-galactosidase deficiency; ceramide trihexoside accumulates.
   f. **Tay-Sachs disease** is due to hexosaminidase A deficiency; GM_2-ganglioside accumulates.
2. **Pathology.** All of these disorders are characterized by **distention of the reticuloendothelial cells** with stored material, resulting in neuronal enlargement and dysfunction, hepatosplenomegaly, and marrow infiltration.
3. **Clinical features** are usually characterized by delayed development with progression to mental retardation and motor impairment.

B. **Mucopolysaccharidoses** are enzymatic defects that lead to the accumulation of glycosaminoglycans in the brain and viscera, often with concomitant excretion of accumulated material in urine.

1. **Specific diseases**
   a. **Hurler's syndrome** is a defect in α-L-iduronidase.
   b. **Hunter's syndrome** is an X-linked recessive defect in iduronosyl sulfatase.
   c. **Sanfilippo syndrome** is caused by a defect in either heparan sulfatase or N-acetylglycosaminidase.
2. **Clinical features** include combinations of mental retardation, hepatosplenomegaly, skeletal changes, and corneal clouding.

Aminoacidurias

1. **Phenylketonuria** is a defect in phenylalanine hydroxylase. Mental retardation may develop, but it can be prevented by a diet low in phenylalanine.
2. **Homocystinuria** is due to a defect in cystathionine synthetase. Clinically multiple thromboembolic infarcts may occur. Restriction of dietary methionine together with pyridoxine supplementation may be helpful.
3. **Maple syrup urine** disease is a defect in branched-chain ketoacid decarboxylase. Mental retardation, seizures, and death occur before the second year of life.

D. **Carbohydrate metabolism disorders**

1. **Hypoglycemia** is defined as low serum glucose.
   a. **Incidence.** It usually occurs in diabetics on insulin, but it is also seen with insulinomas and chronic liver disease.
   b. **Clinical features.** Hypoglycemia causes encephalopathy with delirium or coma, stroke-like deficits, and seizures.
   c. **Pathology.** Focal, diffuse, or laminar cortical necrosis may be evident. The cerebellum may show loss of Purkinje cells.
2. **Familial myoclonic epilepsy (Lafora's disease)** is a rare autosomal recessive disorder. It causes mental deterioration, seizures, and myoclonus. Intraneuronal inclusions (Lafora bodies, which are complex sugar polymers) may be present.

E. **Other metabolic disorders**

1. **Reye's syndrome**
   a. **Incidence.** This syndrome occurs sporadically in children **following viral illnesses** (frequently varicella), usually in association with **aspirin ingestion.**
   b. **Clinical features** include acute fever, vomiting, seizures, altered consciousness leading to coma, and altered liver function with hyperammonemia.
2. **Wilson's disease** is an autosomal **disorder of copper metabolism.**
   a. **Clinical features** include movement disorders, personality change, dysarthria, and liver dysfunction.

CLINICAL CORRELATE

Screening for phenylketonuria is done perinatally. If the disease is discovered early, its manifestations may be prevented by restricting the dietary intake of phenylalanine. If not, the patient will probably need to be institutionalized.

CLINICAL CORRELATE

Reye's syndrome is seen most frequently in children given aspirin after a recent varicella (chickenpox) or influenza infection.

NUTRITIONAL AND TOXIC DISORDERS

A. **Thiamine (vitamin B_1) deficiency.** Thiamine is crucial to cellular energy production. Deficiency is due to dietary insufficiency; in the United States, deficiency is usually due to the malnutrition of **chronic alcoholism.** Two neurologic diseases result:

1. **Beriberi peripheral neuropathy** is an axonal degeneration with secondary demyelination. It is caused by an unknown mechanism.
2. **Wernicke's encephalopathy** is characterized by confusion, ocular disturbance, and ataxia of gait.

B. **Vitamin B_{12} deficiency** is almost always secondary to **malabsorption** rather than to dietary deficiency (except in strict vegetarians). The most common cause is **pernicious anemia,** which results in pathology in the peripheral and optic nerves as well as in the spinal cord and brain.

C. **Ethanol.** Pathology is usually the result of nutritional deficiency, hypoxia, or hepatic disease. The following diseases may accompany alcoholism.

1. **Alcoholic cerebellar degeneration** causes cerebellar atrophy, predominantly in the anterior superior vermis, particularly affecting the Purkinje cells. It occurs mainly in men and is characterized by severe ataxia of the lower extremities.
2. **Central pontine myelinosis** is a condition of localized pontine demyelination. It may also be seen in severe malnutrition and with sudden shifts in serum sodium. It may be asymptomatic or cause severe brain stem dysfunction, quadriparesis, coma, and death.
3. **Alcoholic polyneuropathies** are characterized by axonal degeneration of peripheral nerves, gradual development of paresthesias, weakness, and pain.
4. **Fetal alcohol syndrome (FAS)** describes fetal damage resulting from alcohol use during pregnancy. The amount required to produce the syndrome remains controversial. Features of the syndrome include mental retardation, microcephaly, incoordination, hypotonia, irritability, hyperactivity, and a characteristic facies.

D. **Methanol intoxication** produces **metabolic acidosis** (with an anion gap) and **visual disturbances.** The metabolism of methanol to formaldehyde (and less so, to formic acid) is responsible for the ocular toxicity.

IN A NUTSHELL

Neurological sequelae of thiamine deficiency:

- *Beriberi: peripheral neuropathy*
- *Wernicke-Korsakoff: memory, gait, and eye movement disturbances*

IN A NUTSHELL

Vitamin B_{12} deficiency results in CNS and PNS pathology due to both demyelination and axonal degeneration.

CLINICAL CORRELATE

Methanol poisoning is treated with ethanol; ethanol competes with methanol for alcohol dehydrogenase and prevents the formation of formaldehyde.

INFECTIOUS DISORDERS

A. **Bacterial infections**

1. **Acute pyogenic meningitis**
   a. **Pathogenesis.** Infectious agents are relatively specific to the patient's age, although overlap occurs.
      (1) For neonates, *Escherichia coli*, Group B Streptococci and *Listeria monocytogenes* predominate.
      (2) For infants and children, *Streptococcus pneumoniae* is the most common infectious cause in those that have received the vaccine for *H. influenzae*. *H. influenzae* will predominate in nonvaccinated children.
      (3) In young adults, *Neisseria meningitidis* causes the most cases.
      (4) In the middle-aged and elderly, Pneumococcus predominates.
   b. **Clinical features** include fever, malaise, headache, nuchal rigidity, photophobia, and altered mental status.

2. **Brain abscesses** are localized, walled-off areas of intraparenchymal purulent exudate.
   a. **Etiology.** Brain abscesses may result from:
      (1) Extension of otitis, mastoiditis, or sinusitis
      (2) Contamination of surgical wounds
      (3) Penetrating head injuries
      (4) Hematogenous dissemination from infected heart and lung sites
   b. **Clinical features.** There may be focal or generalized signs. Death follows herniation or, more rarely, rupture with ensuing meningitis and ventriculitis.
   c. **Pathogenesis.** Common organisms include anaerobic Streptococci, Staphylococci, Bacteroides, Gram-negative Bacilli and, less often, Nocardia and Citrobacter.

3. **Neurosyphilis** (tertiary syphilis) follows an asymptomatic meningitis.
   a. **Meningovascular syphilis** involves infiltration of meninges and vessels with chronic inflammatory cells; the resultant arterial fibrosis and infarction is responsible for many of the clinical manifestations, such as hydrocephalus and cranial nerve palsies.
   b. **General paresis** is characterized by meningeal fibrosis and atrophy; it is most severe in the frontal and temporal lobes. Histologic examination reveals neuronal loss, astrocytosis, rod-shaped microglial cells, and spirochetes.

NOTE

Meningitis is an inflammation of the meningeal layers, not the brain parenchyma.

IN A NUTSHELL

Cerebral abscess is a localized parenchymal infection walled off from the rest of the brain. Symptoms may be focal or generalized.

IN A NUTSHELL

Tertiary syphilis involves the meninges and brain parenchyma as well as the spinal cord (tabes dorsalis).

c. **Tabes dorsalis** involves thoracolumbosacral chronic meningeal inflammation with initial injury to dorsal roots and secondary demyelination of the dorsal columns.

4. **Neurotuberculosis** results from hematogenous dissemination of tuberculosis. It is characterized by a thick exudate at the base of the brain or over the dorsal surface of the spinal cord.

B. **Mycotic infections** occur commonly in patients with neoplasia, immunosuppression, and organ transplants.

NOTE

Candida frequently causes infections in immunocompromised hosts.

1. **Candidiasis** is the most often encountered fungal infection at autopsy of the CNS. Virtually all cases result from hematogenous dissemination from distant sites in colonized patients. Lesions are composed of multiple small abscesses. The organisms appear as a **mixture of yeast and pseudohyphae,** a pathognomonic characteristic of Candida.

IN A NUTSHELL

Aspergillosis lesions of the CNS result from hematogenous spread. Aspergillosis tends to invade vascular walls and cause cerebral infarction.

2. **Aspergillosis** is the second most common fungal infection of the CNS encountered at autopsy. It results from initial infection through **inhalation** of **airborne spores with hematogenous dissemination to the brain.** Thus, these infections rarely occur without overt infection elsewhere, especially in the lung.

3. **Mucormycosis** may occur as a regional infection involving the nose, sinuses, and brain (as in **uncontrolled diabetes**), or as a systemic disease with hematogenous dissemination in compromised hosts. Lesions include purulent meningitis, cerebritis, and infarction secondary to arterial invasion and thrombosis.

4. **Cryptococcosis.** Approximately 50% of cases occur in immunocompetent individuals. Inhalation of spores, followed by hematogenous spread, leads most often to meningitis.

C. **Parasitic infections**

1. **Toxoplasmosis**

IN A NUTSHELL

T. gondii is acquired through contact with cats or infected meat. It is an important cause of CNS infection in both host and fetus (since it can cross the placenta).

a. **Acquired toxoplasmosis** is caused by the protozoan *Toxoplasma gondii.* Acutely, there is destruction caused by intracellular, crescent-shaped trophozoites. Chronically, intracellular cysts containing organisms are formed and may remain viable in brain and muscle for years. Normal or immunocompromised adults acquire the organism by consumption of poorly cooked meat or by contamination with feces from infected cats. In severe cases, the brain is littered with multiple large foci of necrosis and many encysted organisms and free trophozoites.

b. **Congenital toxoplasmosis** complicates nearly 40% of primary infections in pregnancy. A maternal infection during the second to sixth month of gestation may result in infant

convulsions, intracerebral calcification, hydrocephalus, and chorioretinitis.

). Viral infections

1. **Meningoencephalitis** may be caused by many agents, but the morphologic features are similar.
   a. **Pathology.** The brain is edematous, containing focal areas of necrosis and even hemorrhage.
   b. **Enteroviruses** are common causes of viral CNS infections. Prior to immunization, poliomyelitis was a prominent example. Today, coxsackievirus (especially group B) and echoviruses are common.
   c. **Arenaviruses** are associated most commonly with lymphocytic choriomeningitis.
   d. **Arboviruses** produce Eastern, Western, Venezuelan, St. Louis, Japanese, and California encephalitis. The St. Louis variety is common in older individuals and carries a mortality of 20%.
   e. **Paramyxoviruses**
      (1) **Mumps** causes meningitis in nearly 25% of patients. A rare meningoencephalitis that appears to be immune-mediated may occur.
      (2) **Measles** (rubeola) is responsible for many cases of postinfectious encephalomyelitis and, in a rare, persistent form, **subacute sclerosing panencephalitis (SSPE).** SSPE mainly affects children. With regard to the pathogenesis of SSPE, damage is caused by an immune reaction to the measles virus.
   f. **Rubella virus** causes the congenital rubella syndrome: low birth weight, cardiac defects, cataracts, chorioretinitis, and neurologic abnormalities.
   g. **Rabies virus** causes pain and paresthesia at the original wound, followed by abnormal behavior, hyperactivity, autonomic dysfunction, and laryngeal muscle spasm. **Negri bodies** (eosinophilic, oval cytoplasmic inclusions) are characteristic.
   h. **Herpesvirus**
      (1) **Herpes simplex** is the most common cause of **sporadic encephalitis,** which can be fatal if untreated. Pathologic changes have a predilection for the frontal and temporal lobes.
      (2) **Varicella zoster** produces a benign cerebellar ataxia and postinfectious encephalomyelitis. The latter entity is characterized by perivenular demyelination. Latent virus may persist in sensory ganglia; reactivation leads to **herpes zoster (shingles),** which commonly affects thora-

IN A NUTSHELL

Negri bodies = rabies

IN A NUTSHELL

Herpes encephalitis is often a result of HSV-1 infection. It is treated with acyclovir.

columbar dermatomes and the ophthalmic division of the trigeminal nerve.

(3) **Cytomegalovirus** may involve the cerebrum with disseminated "glial nodules" that consist of nodular infiltrates of histiocytes; these are usually seen in immunosuppressed individuals.

2. **Transmissible subacute spongiform encephalopathy** is thought to be caused by virus-like agents (prions) with a very long incubation period ("slow viruses").
   a. **Creutzfeldt-Jakob disease (CJD)**
      (1) **Clinical features** include personality changes, incoordination, myoclonus, dementia, and death (within 3 years).
   b. **Kuru** affects tribal people of the mountains of Papua, New Guinea. The incidence has greatly diminished since the practice of cannibalism has ceased.
      (1) **Clinical features** include ataxia, tremor, and death (within 1 year). Dementia is not prominent.

BRIDGE TO IMMUNOLOGY

Prions are proteins without a nucleic acid component. They arise by variable or alternate folding of a particular class of normal proteins. This variable folding causes them to "crystallize" easily, producing plaques. The variable folding pattern is very stable, so these proteins appear infectious, surviving the gastrointestinal tract if ingested and causing normal proteins to "crystallize" with them when they are absorbed into the brain.

NEUROPATHOLOGY OF AIDS

The majority of autopsied AIDS cases have pathologic findings in the neuromuscular system, including primary findings of HIV infections and secondary abnormalities due to complicating conditions. These patients may have abnormalities in one or many structures, including the peripheral nerves, meninges, and the CNS (brain, spinal cord, or both).

A. **Peripheral nerve abnormalities in AIDS**

1. **Incidence.** The actual incidence of peripheral neuropathy associated with HIV seropositivity remains controversial. Symptoms are usually present early in the course of the disease.
2. **Types**
   a. **The most common neuropathy** is demyelinating and segmental with endoneurial inflammatory infiltrates. Motor symptoms are more prominent than sensory.

B. **Meningeal abnormalities in AIDS**

1. **Incidence.** Abnormalities of the meninges in AIDS are usually secondary to other syndromes (including cytomegalovirus infection, neurosyphilis, toxoplasmosis, cryptococcosis, and bacterial infection).

C. **Spinal cord abnormalities in AIDS**

1. **Incidence.** Approximately 25% of HIV-positive individuals have a vacuolar myelopathy on autopsy. This is the most common

finding, but other patients have evidence of inflammation secondary to viral infection (usually herpes or CMV).

D. **Brain abnormalities in AIDS**

1. **Incidence.** Up to 75% of HIV-positive individuals have some form of diffuse neuropathology in the brain. Many AIDS patients are demented, albeit some just mildly, at the time of death. There are, however, several different forms of neuropathology that are associated with dementia.

2. **Types**
   a. **AIDS dementia/encephalitis with multinucleated cells (MNCs).** This is the neuropathology most directly related to AIDS. The clinical course is characterized by a progressive dementia. Neuropathology is remarkable for direct evidence of infection of macrophages and microglia with the HIV virus, destruction of white matter, and **multinucleated cells** (likely comprised of microglia and other cells). MNCs are considered by some to be pathognomic of HIV encephalitis. Simultaneous infection with CMV is extremely common and is probably a contributory cause to the neuropathology. The neurons themselves do not appear to be directly infected by the HIV virus.
   b. **AIDS dementia without MNCs** accounts for approximately 50% of the identified CNS pathology; only a minority of these brains demonstrate direct HIV invasion.
   c. **Lesions associated with AIDS**, but not directly caused by the HIV virus, may be infectious or noninfectious. Noninfectious diseases include primary CNS lymphoma, infarcts secondary to vasculitis, embolic infarcts (sometimes secondary to a peripheral infection), and Kaposi's sarcoma. Infectious diseases include toxoplasmosis, mycobacteria, herpesvirus, CMV alone, and the JC virus (leading to progressive multifocal leukoencephalopathy). Less common agents include various fungi (e.g., Aspergillus, Candida) and Cysticercus.

IN A NUTSHELL

Most HIV-positive patients develop a diffuse CNS pathology that is directly HIV-related; they are also susceptible to focal opportunistic infections.

IN A NUTSHELL

Multinucleated cells in AIDS dementia/encephalitis are the result of HIV infection of the brain microglial cells (monocyte derivatives). CMV infection may contribute as well.

Male Reproductive Pathology

The most common clinical findings in male reproductive pathology are solid tumors of the testes, prostate, and penis. Prostate carcinoma, in fact, is the second most common cause of cancer in men. Since the majority of the tumors are well differentiated and respond to chemotherapy, radiation therapy, or to surgery, it is important to be able to identify the tumors early on in the course of the disease, through the use of screening tests, tumor markers, and clinical signs and symptoms. This chapter will focus on these diseases, as well as on other disorders that commonly affect the male reproductive system.

TESTES

A. **Cryptorchidism**

1. **Clinical features.** Cryptorchidism is the **failure of normal descent of intra-abdominal testes** into the scrotum. It affects approximately 1% of the adult male population, more often on the right side than the left, and may be unilateral or bilateral. Bilateral cryptorchidism can cause infertility by elevating body temperature to a level that interferes with spermatogenesis. Maldescended testes are also susceptible to trauma, and have a greatly increased incidence of testicular cancer.
2. **Pathogenesis.** Cryptorchidism may be a mechanical, hormonal, or idiopathic congenital anomaly.

B. **Germ cell tumors.** Approximately 95% of testicular tumors are germ cell tumors. They are the **most common malignancy in men 15-34** years of age. Although its etiology is unknown, cryptorchidism increases the likelihood of malignancy 14-fold. Infection, trauma, and genetic factors also influence incidence.

Generally, germ cell tumors begin as a painless testicular enlargement, with potential for widespread dissemination, usually via the lymphatics to iliac and para-aortic nodes See summary in table 7-1.

Tumor	Presentation	Pathology
Germ cell		
Seminoma	Radiosensitive	Bulky and homogeneous
Embryonal carcinoma	Often metastatic	Hemorrhagic nodules
Choriocarcinoma	Highly malignant; elevated HCG	Cyto- and syncytiotrophoblast
Yolk sac tumors	Infants and children; elevated AFP	Nonencapsulated
Teratoma	Mature or immature	Variety of tissues
Nongerm cell		
Interstitial (Leydig) cell tumors	May produce estrogens or androgens	Usually unilateral
Sertoli cell tumors	May produce estrogens or androgens	Usually unilateral
Lymphoma	Older men	Often disseminated

Table 7-1. Testicular tumors.

C. **Nongerm cell tumors**

1. **Interstitial (Leydig) cell tumors**
   a. **Clinical features.** Leydig cell tumors can produce androgens, estrogens, or corticosteroids. In children, they often present with masculinization or feminization and in adults with gynecomastia.
   b. **Course and prognosis.** They are usually benign and only 10% are invasive. Surgery may be curative.
2. **Sertoli cell tumors**
   a. **Clinical features.** Sertoli cell tumors can produce small amounts of androgens or estrogens, but usually not enough to cause endocrinologic changes. They may present with testicular enlargement.
   b. **Course and prognosis.** Over 90% of these tumors are benign.
3. **Lymphomas** are the most common testicular cancer in elderly men. The tumors are rarely confined to the testes.

D. **Inflammatory lesions**

1. **Mumps**
   a. **Clinical features.** Orchitis develops in approximately 25% of patients over age 10, usually within a week after parotid swelling. Orchitis is less common in patients under 10 years old.
   b. **Pathology**
      (1) Mononuclear inflammatory infiltrates predominate in the acute phase.
      (2) Atrophy is rare.
   c. **Course.** Mumps rarely leads to sterility. However, should atrophy follow, it may predispose patients to testicular neoplasm.
2. **Gonorrhea**
   a. **Clinical features.** A neglected urethral gonococcal infection may spread to the prostate, to the seminal vesicles, and to the epididymis, but rarely to the testes.
   b. **Pathology.** There may be purulent inflammation or abscesses.
3. **Syphilis**
   a. **Clinical features.** Acquired or congenital syphilis may involve the testes.
   b. **Pathology.** There are two forms: gummas or a diffuse interstitial/lymphocytic plasma cell infiltrate.
   c. **Course.** Syphilis can lead to sterility; Leydig cell involvement can cause a loss of libido.
4. **Tuberculosis**
   a. **Clinical features.** TB usually spreads from the epididymis; this is almost always associated with foci of TB elsewhere.
   b. **Pathology.** Caseating granulomata are the classic finding, as with TB dissemination elsewhere.
5. **"Nonspecific"** inflammation is usually caused by *Chlamydia trachomatis* spread from the epididymis.

E. **Torsion**

1. **Clinical features.** Twisting of the spermatic cord may compromise both arterial supply and venous drainage. Torsion may be precipitated by sudden movement, trauma, or congenital anomalies.
2. **Course.** If not surgically corrected early, torsion may result in testicular infarction with resultant loss of function.

PROSTATE

A. **Benign prostatic hyperplasia (BPH)**

1. **Clinical features.** Benign prostatic hyperplasia (BPH) is characterized by the formation of large nodules in the periurethral region (median lobe) of the prostate, which may narrow the urethral canal to produce varying degrees of urinary obstruction. Patients often present with **difficulty urinating.** It is increasingly common after age 45, and the incidence increases steadily with age (e.g., 90% of men after age 70).
2. **Pathogenesis.** The mechanism is poorly understood, but blocking androgens seems to reduce the incidence.
3. **Course.** The course can follow an asymptomatic pattern, or it could result in urinary symptoms and urinary retention, leading to secondary bladder changes, such as smooth muscle hypertrophy. Currently, it is thought that this disease does not predispose to prostatic carcinoma.

B. **Carcinoma**

1. **Clinical features.** Prostatic carcinoma is the second most common cancer in men. Prostate cancer usually occurs after age 50, and the incidence increases steadily with age. In addition to clinically significant tumors, a high incidence of small "incidental" or "latent" carcinomas is discovered at autopsy in men over age 50. The disease may present with urinary problems or a palpable mass on rectal examination.
2. **Pathogenesis** is unknown. The tumor is age related, associated with race (more common in African-Americans than in Caucasians, relatively rare in Asians), and is under endocrinologic and environmental influences.
3. **Pathology**
   a. **Grossly,** prostate cancer is found usually in peripheral tissue and is firm and "gritty" as a result of fibrosis.
   b. **Microscopically,** most are **adenocarcinomas.**
4. **Course and prognosis**
   a. **Staging of prostatic tumors** depends upon the size of the tumor, the degree of infiltration to local tissues, and the degree of metastasis.
   b. **Metastases** may occur via the lymphatic or hematogenous route; bone is commonly involved with **osteoblastic metastases,** typically in the pelvis and lower vertebrae.
   c. **Tumor markers. PSA (prostate-specific antigen)** is an enzyme produced by normal, hyperplastic, and malignant prostate glands. It is elevated in both hyperplasia and can-

NOTE

The "H" in BPH more accurately represents hyperplasia than hypertrophy, although you may see either term still used.

CLINICAL CORRELATE

The urinary symptoms of BPH include frequency, nocturia, and problems initiating and stopping the urinary stream.

NOTE

While prostate cancer is more common than lung cancer, mortality from lung cancer is more than twice the mortality from prostate cancer.

CLINICAL CORRELATE

An elderly man with osteoblastic metastases visible on x-ray should be considered to have prostate carcinoma until proven otherwise.

cer, but usually more so in the latter. Elevated PSA together with an enlarged prostate on digital rectal exam is highly suggestive of carcinoma.

d. **Survival** is correlated with stage and grade. Unfortunately, most patients present with advanced disease and have a 10-year survival rate of less than 30%.

e. **Treatment** involves surgery, radiation, and hormonal modalities (orchiectomy and estrogenic drugs).

C. **Infections**

1. **Acute prostatitis**

a. **Clinical features.** This condition usually results from a bacterial infection of the prostate. Pathogens are often organisms that cause urinary tract infection. ***Escherichia coli*** is the most common, followed by other Gram-negative rods. Infection may follow manipulation of the prostate or urethra (e.g., cystoscopy, catheterization).

b. **Pathogenesis.** Bacteria spread by direct extension from the posterior urethra or the bladder. Lymphatic or hematogenous spread can also occur.

c. **Course.** Appropriate antibiotic therapy is usually curative.

2. **Chronic prostatitis**

a. **Clinical features**. Chronic prostatitis is a common cause of recurrent urinary tract infections in men. There are two types: bacterial and nonbacterial. Both forms may be asymptomatic or may present with lower back pain and urinary symptoms.

b. **Pathogenesis.** Bacterial infection may be a sequela of acute prostatitis; nonbacterial infections are most often caused by Ureaplasma and *Chlamydia trachomatis.*

PENIS

A. **Congenital malformations**

1. **Hypospadias and epispadias**

a. **Clinical features.** These are malformations of the urethral groove and canal. They are often associated with cryptorchidism and other congenital anomalies.

b. **Pathology.** In hypospadias, the urethra opens onto the ventral surface of the penis. In epispadias, the urethra opens onto the dorsal surface.

c. **Course.** Both of these malformations may cause infertility.

2. **Phimosis.** In this condition, the prepuce orifice is too small to be retracted normally. It interferes with hygiene, and can also predispose to bacterial infections.

B. **Infections**

1. **Condyloma acuminatum**
   a. **Clinical features.** This is a benign lesion of **papillomavirus** origin, which may occur on any mucous membrane.

2. **Syphilis.** Syphillis is a sexually transmitted or congenital disease caused by the spirochete ***Treponema pallidum.*** The acquired form initially presents with cutaneous manifestations followed by widespread dissemination. The disease occurs in stages.
   a. **Primary syphilis** has an average 3-week incubation during which spirochetes spread throughout the body. Painless **chancre sores** form and heal within 1-3 months.
      (1) **Grossly,** chancres usually occur on the glans penis in men and the vulva or cervix in women. Occasionally, they appear on other mucous membranes, such as the oral cavity. They appear as single, firm, red papules that may ulcerate.
      (2) **Microscopically,** there is a mononuclear infiltrate with obliterative endarteritis. Plasma cells are very prominent. Special stains may show treponemes.
   b. **Secondary syphilis** usually develops 1-2 months after the primary stage. There is a **local or generalized rash** lasting 1-3 months. The rash can involve the palms and soles as well as mucous membranes. **Condyloma lata** can appear on the penis.
      (1) **Grossly,** condyloma lata are flat, brown red papules on the penis or other mucous membranes. They may form large plaques or ulcerate.
      (2) **Microscopically,** there is an obliterative endarteritis and plasma cell infiltrate. Treponemes are present.
   c. **Tertiary syphilis** develops in one-third of untreated patients. It may affect the central nervous system (CNS), heart, and skin. **Neurosyphilis** has a varied presentation, including meningovascular, tabes dorsalis, and general paresis. CNS gummas are rare. An **obliterative endarteritis** can occur, involving the vasa vasorum of the aorta, which can lead to the formation of an **aneurysm.** Elastic stains show destruction of fibers. In other tissues the characteristic lesion is the **gumma.**
      (1) **Grossly,** gummas may be single or multiple. They have rubbery, necrotic central focus that is variable in size. They are most common in the liver, testes, and bone.
      (2) **Microscopically,** gummas are composed of **mononuclear cells surrounding a center of coagulative necrosis.** Gummas resemble caseating granulomas but usually do

not have multinucleated giant cells and do not have any stainable acid-fast organisms. Treponemes are rare.

d. **Serologic tests for syphilis**

(1) **VDRL** tests for nonspecific antibodies evoked by spirochetal infection; these antibodies react with **cardiolipin,** a lipoid substance from beef heart. The VDRL becomes positive a few weeks after primary infection and may remain persistently elevated. **"False positives"** may occur in many acute illnesses, as well as in chronic mononucleosis, leprosy, hepatitis, and autoimmune disorders.

(2) **Fluorescent treponemal antibody (FTA)** tests for specific spirochetal antigens. It can confirm the presence of active syphilis in a patient with a positive VDRL.

3. **Chancroid**

a. **Clinical features.** This is a sexually transmitted infection caused by *Haemophilus ducreyi.* Patients have a **painful chancre** and regional **lymphadenopathy.**

4. **Lymphogranuloma venereum (LGV)**

a. **Clinical features.** LGV is a sexually transmitted disease caused by *Chlamydia trachomatis.* It is rare in the United States but common in tropical areas. LGV can present with genital or anorectal lesions or regional lymphadenopathy.

b. **Course.** Scarring and fibrosis can follow chronic infection. Subsequent lymphatic obstruction can lead to edema and elephantiasis of the lower extremities and external genitalia.

5. **Genital herpes**

a. **Clinical features.** There are two subtypes: **herpes simplex (HSV I)** and **herpes genitalis (HSV II),** causing an overlapping spectrum of disease.

b. **Pathology**

(1) **Grossly,** HSV I and II cause vesicles on the external genitalia and mucous membranes; ulcerations can also develop.

(2) **Microscopically,** epithelial cells show cytopathic changes. Cell fusion leads to **giant cells** or polykaryons, which can be seen on a smear of blister fluid or **Tzanck smear.**

c. **Course.** HSV infections **tend to recur.** The virus can remain latent in nerve ganglia. **Acyclovir** may prevent or decrease the frequency of recurrences.

NOTE

Chancroid is not caused by the same etiologic agent as chancre (syphilis). Chancroid is caused by H. ducrei, while chancre is caused by T. pallidum.

CLINICAL CORRELATE

HSV I usually appears above the waistline; HSV II typically appears below.

C. **Carcinoma**

1. **Bowen's disease** is **carcinoma in situ** and can be associated with visceral malignancy. It usually occurs in men or women over age 35. In men, it tends to involve the shaft of the penis and the scrotum.

2. **Squamous cell carcinoma**

   a. **Clinical features.** Squamous cell carcinoma of the penis accounts for 1% of cancers in men in the United States, usually age 40-70. Circumcision decreases the incidence. **Human papilloma virus infection** is closely associated with the development of squamous carcinoma.

   b. **Course and prognosis.** Squamous carcinoma is usually slow growing and nonpainful; patients often delay seeking medical attention. Metastases can go to local lymph nodes. The prognosis depends on the degree of advancement of the tumor; limited have a greater than 90% 5-year survival rate.

NOTE

Squamous cell carcinomas, particularly those associated with papilloma viruses, show a markedly higher incidence in HIV infection. Its configuration is often papillary. Patients can also have perianal carcinomas or papillomas.

Papillomaviruses (types 16 and 18) are most often associated with squamous carcinoma.

Female Reproductive Pathology

Each organ in the female reproductive system is susceptible to specific disease states, including cancers, benign hyperplasia, and infection. Gynecologic malignancies account for a large majority of cancers in women: breast cancer is the second leading cause of cancer death in women in the U.S., and cervical cancer is the sixth leading cause. Since many gynecologic disorders present with similar symptoms of menstrual irregularities, nonmenstrual vaginal bleeding or discharge, and pelvic pain, it is important to be able to recognize the features that differentiate one disease state from another. This chapter will focus on these disorders and will highlight the risk factors and clinical and pathologic features that distinguish them.

UTERUS

A. **Endometrium**

1. **Endometritis**
   a. **Acute endometritis** is relatively uncommon. It is caused by bacterial infection of the endometrium, usually **following childbirth or abortion.** It may be related to retained products of conception. The usual pathogens are group A β-hemolytic Streptococci and Staphylococci, producing a nonspecific interstitial inflammation with neutrophils.
   b. **Chronic endometritis** may occur postabortion or postpartum but is also related to the use of intrauterine devices (IUDs), tuberculosis (TB), or pelvic inflammatory disease (PID). There is a chronic inflammatory infiltrate with plasma cells in the interstitium.

2. **Endometriosis**
   a. **Clinical features.** Endometriosis refers to **ectopic endometrial tissue outside the uterine cavity,** most often in the ovaries, uterine ligaments, rectovaginal septum, pelvic peritoneum, postlaparotomy scars, vagina, vulva, and appendix. It occurs in women of reproductive age and is rare over age 50. It is commonly associated with infertility. Depending on the location of ectopic tissue, it may cause dysmenorrhea, pelvic pain, dyspareunia, pain on defecation, dysuria, and an inability to conceive.
   b. **Course and prognosis.** Infertility may result from fibrosis and scarring of tubes and ovaries.

NOTE

Endometriosis is characterized by cyclic bleeding from the ectopic endometrial tissue, resulting in "chocolate cysts"—brown blood-filled spaces.

3. **Endometrial hyperplasia**
   a. **Clinical features.** Hyperplasia may occur in both perimenopausal and postmenopausal women. It may present with abnormal or excessive uterine bleeding, often associated with states of **elevated estrogen production** (e.g., prolonged estrogen therapy, adrenocortical hyperfunction, certain ovarian tumors). It may be a precancerous lesion.

NOTE

In postmenopausal women, endometrial hyperplasia is often due to exogenous estrogen administration. Occasionally, well-differentiated adenocarcinoma arises in this setting. It regresses when estrogen therapy is stopped.

4. **Endometrial polyps**
   a. **Clinical features.** These are most common in perimenopausal or postmenopausal women. They usually present with **uterine bleeding.**
   b. **Pathology**
      (1) **Grossly,** polyps may be single or multiple and are usually less than 3 cm. They are often sessile but may be pedunculated.
      (2) **Microscopically,** there are two types: functional endometrium and hyperplastic (cystic or adenomatous) endometrium.
   c. **Course and prognosis.** Malignant transformation is rare.

5. **Endometrial carcinoma**
   a. **Clinical features.** This disease primarily occurs in the postmenopausal age group. It is associated with obesity, diabetes, hypertension, and infertility. It may be asymptomatic or present with abnormal uterine bleeding/discharge.
   b. **Pathogenesis** is thought to be the result of **prolonged estrogen stimulation.** There is a correlation with prolonged estrogen therapy, estrogen-secreting neoplasms, and endometrial hyperplasia (which may also be a result of estrogen stimulation).
   c. **Pathology**
      (1) **Grossly,** tumors are either polypoid or diffuse and may become fungating and necrotic.

CLINICAL CORRELATE

Risk factors for endometrial carcinoma:

- *Prolonged estrogen stimulus*
- *Obesity*
- *Diabetes*
- *Hypertension*

(2) **Microscopically,** one sees primarily **adenocarcinomas,** showing glandular patterns with varying degrees of differentiation.

d. **Course and prognosis.** There is early spread by contiguous growth through the myometrium, into the broad ligament, and then to nearby portions of the gastrointestinal and urinary tract. Later, lymphatic and hematogenous spread occur. Survival depends on the stage at diagnosis. Treatment is usually surgery, with or without radiation.

. **Myometrium**

1. **Leiomyoma (fibroids)**
   a. **Clinical features.** These are **benign smooth muscle neoplasms** representing the most common tumor in women and occurring generally in the third and fourth decades. Incidence is increased in African-American women. There is a possible role of estrogens in the pathogenesis. They may present with excessive menstrual bleeding, abnormal uterine bleeding, pain, infertility, or urinary symptoms.
   b. **Course and prognosis.** Malignant transformation is rare. They may require surgical removal for bleeding or infertility.

FALLOPIAN TUBES (UTERINE TUBES, OVIDUCTS)

A. **Inflammation**

1. **Pelvic inflammatory disease (PID)**
   a. **Clinical features.** Inflammation begins in the vulva or accessory glands (Bartholin's glands or Skene's ducts) and may spread upward throughout the reproductive system. The most common organism is **N. gonorrhoeae,** but Staphylococcus, Streptococcus, coliforms, *Clostridium perfringens,* Mycoplasma, Chlamydia, and anaerobes may be seen. PID may occur spontaneously, postabortion, or postpartum. Presentations include abdominal/pelvic pain, menstrual irregularities, dysmenorrhea, and fever.
   b. **Course and prognosis.** Complications are severe and include sepsis, peritonitis, adhesions with intestinal obstruction, and infertility from tubal scarring or tubo-ovarian abscess. Early diagnosis and appropriate antibiotic therapy are essential.

B. **Tumors** in the fallopian tubes are rare.

CERVIX

A. **Inflammation**

1. **Acute and chronic cervicitis**
   a. **Clinical features.** Various forms of cervicitis are common and are of variable clinical significance. The most common pathogens are Streptococcus, Enterococci, *Escherichia coli,* and Staphylococcus; others include Gonococci, *Trichomonas vaginalis,* Candida and Herpes (usually HSV II). Infection may follow intercourse, childbirth, and gynecologic instrumentation. Predisposing influences include high estrogens, alkaline mucus, and congenital anomalies.
   b. **Course and prognosis.** Cervicitis may be asymptomatic or present with a vaginal discharge. It is important to distinguish it from cervical cancer, usually by Pap smear or biopsy.

B. **Tumors**

1. **Cervical polyps**
   a. **Clinical features.** Polyps are common in the fourth and fifth decades. They may present with irregular vaginal bleeding.
   b. **Pathology**
      (1) **Grossly,** polyps are usually single, arise in the endocervical canal, and may be sessile or pedunculated.
   c. **Course and prognosis.** They may be removed by curetage.

2. **Squamous cell carcinoma of the cervix**
   a. **Clinical features.** This is the sixth most common cause of cancer death in women. It may occur at any age from the second decade onward, but it is most common in the fourth and fifth decades.
   b. **Pathogenesis.** There is an increased risk associated with early onset of sexual relations and multiple sexual partners. **Human papillomaviruses,** particularly types 16 and 18, are clearly associated with squamous carcinoma. Other types are associated with benign papilloma. In patients infected with HIV, cervical cancer due to papillomaviruses is increasing in incidence.
   c. **Pathology**
      (1) **Grossly,** advanced, invasive cervical cancer may be fungating, ulcerating, or infiltrative.
      (2) **Microscopically,** koilocytosis and squamous vacuolizations are seen. Cervical cancer is viewed as the end stage of progression of atypia in cervical epithelium, or cervical intraepithelial neoplasia (CIN). Premalignant squamous epithelium begins to show atypical features (pleomorphism, loss of polarity, frequent mitoses, and

CLINICAL CORRELATE

Risk factors for cervical carcinoma:

- *Early sexual activity*
- *Multiple sexual partners*
- *Lower socioeconomic group*

CLINICAL CORRELATE

Now that we appreciate the importance of papillomaviruses as causative agents of cervical cancer, in situ hybridization studies for specific viral DNA may be done on suspicious lesions.

Figure 8-1. Cervical carcinoma in situ (microscopic).

increased nuclear/cytoplasmic ratio), but maintains differentiation in the upper cell layers.

d. **Course and prognosis.** Cervical cancer spreads by contiguous growth to involve urinary structures and bowel. Lymphatic or hematogenous dissemination may occur. Mortality is declining as a result of early recognition of the precursor dysplastic cervical epithelium via the **Pap smear,** which should be a part of every woman's yearly physical examination from reproductive age onward. The cure rate for carcinoma in situ may be as high as 100%. More advanced disease has a much lower cure rate (10%-15% if metastasis has occurred) and may require both surgery and irradiation.

CLINICAL CORRELATE

The recommended frequency of Pap smears is under discussion. Sexually active women should probably have a Pap smear every year. Sexually inactive women may only need a smear every 3 years. Women with suspicious findings should have repeat Pap smears perhaps as often as every 3-6 months. Repeatedly suspicious or positive smears should lead to cervical biopsy for definitive diagnosis.

VAGINA

A. **Congenital anomalies**

1. **Gartner's duct cysts**
   a. **Clinical features.** These cysts must be distinguished from tumor masses.
   b. **Pathology**
      (1) **Grossly,** 1-2 cm submucosal cysts on the lateral vaginal walls are present.
      (2) **Microscopically,** the cysts may have cuboidal, columnar, transitional, or a mixed epithelial lining. There is no atypia.

B. **Inflammation** may be due to various pathogens.

1. ***Trichomonas vaginalis*** is a flagellated protozoan. It typically causes a "strawberry red" vaginal mucosa and microscopic, suppurative inflammation.
2. ***Candida albicans*** (monilia) causes a thick, white exudate.
3. **Herpes simplex vaginitis** may accompany vulvar infection. It may lead to neonatal infection during delivery. The organism is usually herpes simplex virus (HSV) II.
4. **Gonococcus** may cause vulvovaginitis in children as well as adults.

C. **Tumors**

1. **Squamous cell carcinoma**
   a. **Clinical features.** This tumor is quite rare, comprising less than 1% of female genital cancers. It may present with irregular bleeding, spotting, or discharge but is occasionally asymptomatic until advanced.
   b. **Course and prognosis.** Squamous carcinoma of the vagina spreads by local extension to the cervix, rectum, and bladder. Late lymphatic or hematogenous spread may occur.
2. **Adenocarcinoma**
   a. **Clinical features.** Adenocarcinoma is very rare; of the vaginal cancers, 95% are squamous and 5% are adenocarcinoma. It is much more common in young women whose mothers were treated during pregnancy with **diethylstilbestrol (DES).** Because DES is no longer used to treat threatened abortion, this cause of adenocarcinoma has almost disappeared.
   b. **Course and prognosis.** Tumors grow by contiguous spread; later, they grow by lymphatic or hematogenous spread. Treatment includes surgery with or without radiation. DES-related tumors have an 80% 5-year survival.

CLINICAL CORRELATE

DES therapy in pregnant women increases the incidence of vaginal neoplasms in their daughters. Vaginal adenosis is a benign condition that is thought to be a precursor of clear cell adenocarcinoma in these women.

OVARIES

A. **Overview.** The most common ovarian lesions are cysts and tumors. Ovarian cancer is the fifth most common cancer in women, accounting for 6% of all female cancers. Eighty percent of ovarian tumors are benign. The benign tumors tend to occur earlier (in the third through the fifth decades), while the malignant tumors are more common in older women (in the fifth through the seventh decades). **Family history, early menarche,** and **nulliparity** are risk factors. There is an increased incidence in children with gonadal dysgenesis. Tumors are **often asymptomatic until large;** then, they may present with abdominal pain, distension, vaginal bleeding, and gastrointestinal or urinary

symptoms. Ovarian tumors are classified into four groups: surface epithelial tumors, germ cell tumors, sex cord/stromal tumors, and metastatic tumors involving the ovary.

B. **Surface epithelial tumors**

1. **Overview.** 60% of ovarian tumors arise from surface epithelium. There are variable presentations. In addition to abdominal discomfort, vaginal bleeding, and gastrointestinal/genitourinary symptoms, patients may present with malignant ascites from peritoneal seeding or with an acute abdomen from torsion of a large tumor or rupture of a cyst. Unfortunately, these slow-growing tumors are often asymptomatic until the disease is far advanced.

C. **Germ cell tumors**

1. **Overview.** These comprise 15%-20% of all ovarian neoplasms; **most (over 95%) are benign cystic teratomas.** They occur primarily in young women and children. Presentations vary. Germ cell tumors are divided into four groups: teratomas, dysgerminomas, endodermal sinus (yolk sac) tumors, and "other," a group that includes choriocarcinoma, embryonal carcinoma, polyembryoma, and mixed germ cell tumors.

2. **Teratomas**

   a. **Mature (benign) teratomas** are also called **dermoid cysts** because they are lined by skin and adnexae and are **often filled with sebaceous secretions and hair.** There is a high incidence in women of reproductive age. They may present with an abdominal mass, pain, and gastrointestinal or menstrual abnormalities.

      (1) **Grossly,** they are usually unilateral, small, unilocular cysts that may have a mixture of hair, tooth structures, and bone (Figure 8-2).

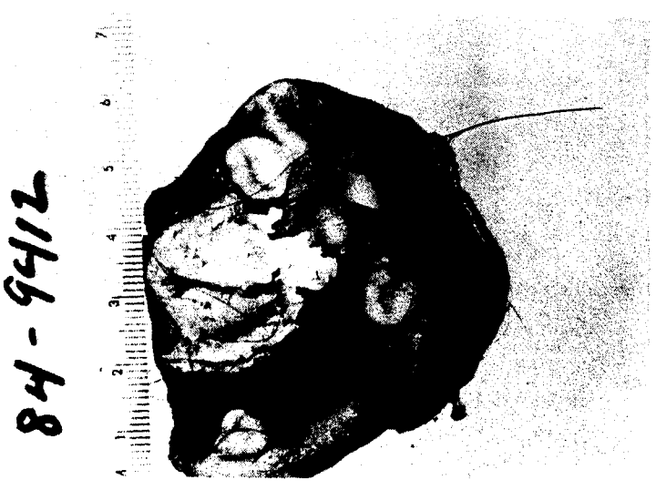


Figure 8-2. Dermoid cyst of the ovary (gross).

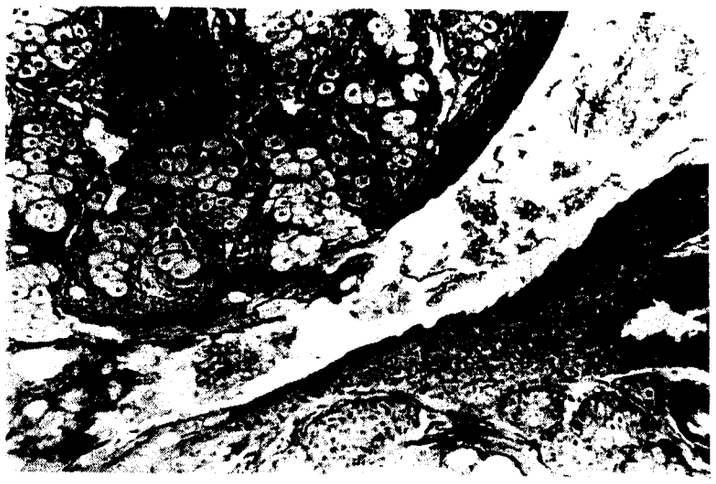


Figure 8-3. Dermoid cyst of the ovary (microscopic).

(2) **Microscopically,** they are usually composed of cysts, lined by stratified squamous epithelium and structures derived from **multiple germ layers,** including endoderm, mesoderm, and ectoderm (Figure 8-3).

(3) The course is usually benign. Surgical resection leads to cure. Torsion may occur.

b. **Immature (malignant) teratomas** are rare, containing embryonic elements derived from all three germs layers.

(1) **Grossly**, they form smooth, large masses that are primarily solid but may have cystic spaces. **Necrosis and hemorrhage are common.** As with the benign tumors, malignant teratomas may contain hair and bone.

(2) **Microscopically,** they are composed of immature tissues differentiating into nerve, muscle, bone, and a variety of other tissues. The characteristic of malignancy is undifferentiated areas.

(3) These tumors are **fast-growing and invasive** with both lymphatic and hematogenous spread. Grading is based on degree of maturity of cells and presence of neural tissue: the more immature the cells and the more neuroepithelium, the higher the grade and the worse the prognosis.

D. **Sex cord-stromal tumors**

1. **Overview.** These tumors comprise only 5%-10% of ovarian neoplasms. They occur at all ages, and **produce steroid hormones** that may lead to endocrinologic syndromes. The three most important groups of these tumors are granulosa-theca cell, fibroma, and Sertoli-Leydig cells.

E. **Tumors metastatic to the ovary.** The most common sites of origin are other pelvic organs, upper gastrointestinal tract, and breast. **Krukenberg's tumor** is bilateral, metastatic, mucin-producing adenocarcinoma (usually signet-ring cells derived from the stomach).

F. **Cysts**

1. **Follicular cysts**
   a. **Clinical features.** These are often asymptomatic cysts, originating in unruptured or resealed follicles.
   b. **Pathology**
      (1) **Grossly,** these cysts are usually in the cortex, multiple, filled with clear fluid, and approximately 1 cm in size.
      (2) **Microscopically,** granulosa lining cells may be identified in cysts with little fluid; with large amounts of fluid, pressure causes atrophy of lining cells.
   c. **Course and prognosis.** These cysts generally remain small. Some may produce estrogens and cause endometrial hyperplasia.

2. **Luteal cysts**
   a. **Clinical features.** These benign cysts are less common than follicular cysts.
   b. **Pathology**
      (1) **Grossly,** cysts are usually approximately 2 cm and yellow.
      (2) **Microscopically,** the cystic lining is luteal tissue, comprised of large cells filled with smooth endoplasmic reticulum (like normal corpus luteum cells).

3. **Polycystic ovaries**
   a. **Clinical features.** Polycystic ovaries may be associated with three clinical syndromes: **virilism; excessive menstrual bleeding;** or the **Stein-Leventhal syndrome,** which is characterized by secondary amenorrhea, obesity, hirsutism, infertility, and bilaterally enlarged, polycystic ovaries. They are most common in young women in the second and third decades.
   b. **Pathology**
      (1) **Grossly,** the ovaries are enlarged, with a thick, white outer covering and multiple cysts.
      (2) **Microscopically,** cysts are lined with granulosatheca cells.
   c. **Pathogenesis** is thought to be an abnormality in the hypothalamopituitary axis: the ovary is continuously stimulated by FSH, and LH -cysts form. Luteinized theca cells make androgens, accounting for the masculinization symptoms.
   d. **Course and prognosis.** Wedge resection (by removing cysts and theca cells) may restore normal menses and fertility.

IN A NUTSHELL

Polycystic ovaries:

- *Occur mainly in young women*
- *Associated with:*
  - *Amenorrhea*
  - *Infertility*
  - *Obesity*
  - *Hirsutism*
  - *↑ LH secretion*

BREAST

A. **Acute mastitis**

1. **Clinical features.** Fissures in nipples during early nursing or from skin disorders may predispose to bacterial infection of the breast. Usual pathogens are ***Staphylococcus aureus*** and Streptococcus.
2. **Pathology.** Mastitis is usually unilateral, with pus in the ducts. Necrosis may occur.
3. **Course.** Antibiotics and surgical drainage may be adequate therapy, but necrosis and subsequent fibrosis of a localized area of breast tissue may occur.

B. **Mammary duct ectasia (plasma cell mastitis)**

1. **Clinical features.** This disorder generally occurs in the fifth decade in multiparous women. It presents with pain, redness, and swelling around the areola.
2. **Pathology.** Involvement is usually unilateral, causing a thickened, indurated area of the breast with thick secretions.
3. **Course.** Skin fixation, nipple retraction, and axillary lymphadenopathy may occur and must be distinguished from malignancy.

C. **Fibrocystic disease (cystic hyperplasia)**

1. **Clinical features.** This is the **most common breast disorder;** it is responsible for 50% of breast surgery, affecting approximately 10% of women. It usually develops during reproductive life and represents a distortion of the normal breast changes associated with the menstrual cycle. Patients often have lumpy, tender breasts.
2. **Pathogenesis** is thought to be due to high estrogen levels, e.g., estrogen therapy, or estrogen-secreting neoplasm, coupled with progesterone deficiency.
3. **Pathology.** Several morphologic patterns are recognized.
   a. **Fibrosis** usually affects women 35-49 years of age and is not premalignant.
   b. **Cystic disease** usually affects women 45-55 years of age and may predispose to malignancy.
   c. **Sclerosing adenosis** usually affects women 35-45 years of age and probably does not predispose to cancer.
   d. **Epithelial hyperplasia** occurs in women over 30 years of age (usually 35-45) and represents an increased cancer risk.

Fibrocystic disease	Breast cancer
Often bilateral	Often unilateral
May have multiple nodules	Usually single
Menstrual variation	No menstrual variation
Cyclic pain and engorgement	No cyclic pain or engorgement
May regress during pregnancy	Does not regress during pregnancy

Table 8-1. Features distinguishing fibrocystic disease from breast cancer.

4. **Course and prognosis.** Fibrocystic disease is clinically important, because it may be mistaken for cancer, and it may predispose to cancer, particularly the epithelial hyperplasia variant. Table 8-1 lists the features that differentiate the two diseases.

D. **Gynecomastia**

1. **Clinical features.** Gynecomastia is an **enlargement of the male breasts** that occurs in various clinical situations (e.g., Klinefelter's syndrome; testicular tumors [particularly Sertoli-Leydig cell tumors], puberty, or old age) and is associated with **increased sensitivity to estrogens** (e.g., in hepatic cirrhosis, the liver cannot properly metabolize estrogens).

E. **Tumors**

1. **Fibroadenoma**
   a. **Clinical features.** This is the **most common benign breast tumor.** It occurs in women of reproductive age, generally before age 30 and may be related to increased estrogen sensitivity. Fibroadenoma presents as a **single movable breast nodule,** not fixed to the skin.
   b. **Pathology**
      (1) **Grossly,** there is a small, freely movable nodule, often in the upper outer quadrant. Size may range up to 10 cm. The tumors are usually round and encapsulated with a grey-white cut surface .
      (2) **Microscopically,** fibroadenomas form glandular epithelial-lined spaces with a fibroblastic stroma. Stromal proliferation may collapse gland lumina, or alternatively, glandular proliferation may predominate with scanty connective tissue stroma. Usually, there is a network of ducts within a proliferated, edematous stroma.
   c. **Course.** Fibroadenomas may show menstrual variation and increased growth during pregnancy. Postmenopausal regression is usual. Surgery is required for definitive diagnosis.

2. **Cystosarcoma phyllodes (Phyllodes tumor)**
   a. **Clinical features.** These are fibroadenoma-like tumors that have become large, cystic, and lobulated. They are distinguished from fibroadenomas by the nature of the stromal component. When hypercellular, the term "cellular

fibroadenoma" is used. When the stroma is both hypercellular and highly atypical, the tumor is called a sarcoma.

3. **Intraductal papilloma**
   a. **Clinical features.** This forms a solitary lesion within a duct or cyst and is most common in women 20-50 years of age. It may present with **nipple discharge** (serous or bloody), nipple retraction, or as a small subareolar mass.
   b. **Course.** Current evidence suggests that single intraductal papillomas are benign, but multiple papillomas are associated with an increased risk of cancer.
4. **Carcinoma of the breast**
   a. **Incidence.** Breast carcinoma is the second most common cause of cancer death in women, surpassed recently by lung cancer. It is rarely seen in women under age 25.
   b. **Etiology.** Risk factors include increasing age (particularly after 40), nulliparity, family history (especially in premenopausal cancer), early menarche/late menopause, fibrocystic disease (especially epithelial hyperplasia), and a previous history of breast cancer. The lifetime risk of breast cancer for the average woman with no family history is 8%-10%.
   c. **Clinical features**
      (1) Locations of breast carcinoma are: 50% in the upper outer quadrant, 20% in central area, and 10% in other quadrants. Ninety percent arise in ductal epithelium, while 10% arise in the lobules. Carcinoma is slightly more common in the left breast (110:100); it is bilateral or sequential in 4% of cases.
      (2) Most patients present with a breast mass discovered either by self-examination or on a routine physical examination by a physician.
      (3) Depending on the size and invasiveness of the tumor, other clinical patterns may occur. The tumor may grow into the thoracic fascia to become fixed to the chest wall; it may extend into the skin, causing **dimpling and retraction;** it may cause obstruction of subcutaneous lymphatics, causing an orange-peel consistency to skin called **"peau d'orange";** or it may invade Cooper's ligaments within ducts to cause **nipple retraction.**
   d. **Pathogenesis** is multifactorial: genetic (e.g., family history), environmental (e.g., radiation), and viral (e.g., mammary tumor virus in mice) influences may be involved. A possible hormonal role has been intensively studied, yielding the finding that "unopposed estrogen" over prolonged periods may lead to ductal hyperplasia and malig-

IN A NUTSHELL

Risk factors for breast cancer:

- *Increasing age (40+)*
- *Nulliparity*
- *Family history*
- *Early menarche*
- *Late menopause*
- *Fibrocystic disease*
- *Previous history of breast cancer*
- *Obesity*
- *High-fat diet*

nant transformation.

e. **Metastatic tumors to the breast** are rare. The most common are leukemia, lymphoma, lung cancer, and melanoma.

f. **Diagnosis. Mammography,** a radiologic evaluation of the breast, is part of the clinical investigation of a breast mass. Sixty percent of breast cancers have foci of calcification (Figures 8-4 and 8-5).

g. **Metastases.** Most breast cancers disseminate via lymphatic or hematogenous routes to axillary, supraclavicular, and

NOTE

The American Cancer Society recommends annual mammography for all women over 40.

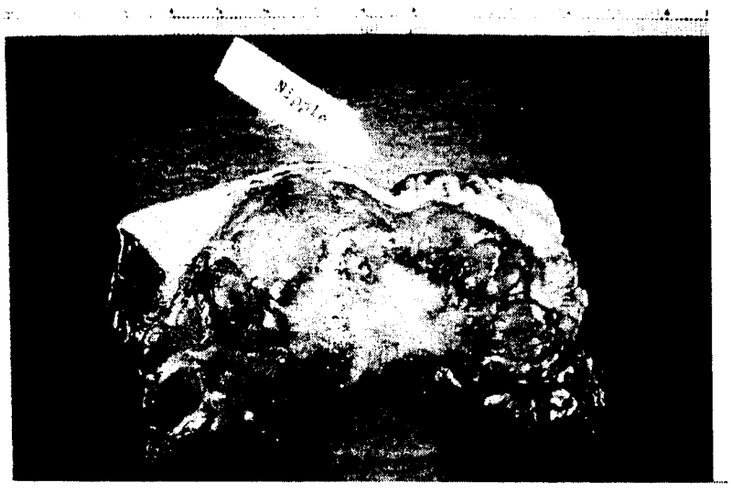


Figure 8-4. Carcinoma of the breast (gross).

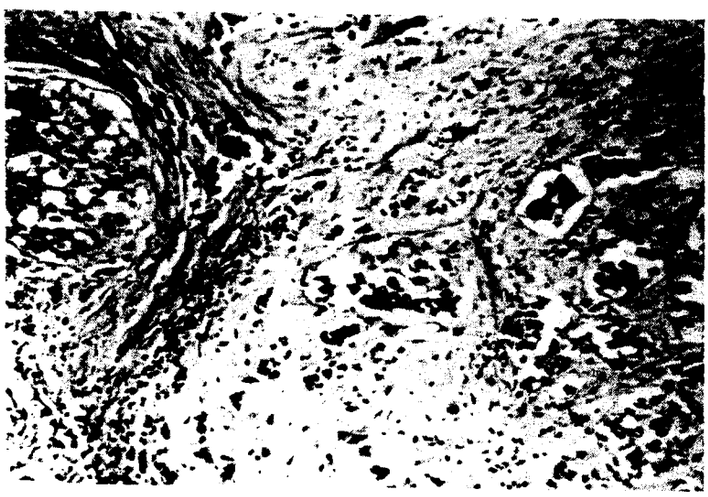


Figure 8-5. Carcinoma of the breast (microscopic)

internal thoracic nodes or the nodes of the contralateral breast. The direction of spread depends on the anatomic location and lymphatic drainage of the primary tumor.

h. **Staging of breast carcinomas** is based upon the size of the tumor and the degree to which the tumor has spread to the surrounding tissues.

i. **Prognosis** depends on many factors, including the type and stage of the carcinoma. Overall 5-year survival is 50%.

j. **Treatment**

(1) **Surgery. Segmental mastectomy** (can be local excision, quadrant excision, partial removal of breast tissue, or "lumpectomy"), where only the tumor and its surrounding tissue is removed. **Simple mastectomy** is the removal of breast tissue; **modified radical mastectomy** is the removal of the breast tissue, axillary nodes, and pectoralis fascia; and **radical mastectomy** is the removal of breast tissue, axillary nodes, pectoralis fascia, and pectoral muscles. Therapy usually includes some **combination of surgery, radiation, and chemotherapy.**

(2) **Estrogen receptors.** The presence of cytoplasmic estrogen receptors may be a useful predictor of response to "hormonal therapy." Approximately two-thirds of breast cancers are estrogen-receptor-positive; most of these will regress when patients are given anti-estrogen compounds such as **tamoxifen.** In fact, patients who are both estrogen- and progesterone-receptor-positive have the best response to tamoxifen. **Adjuvant chemotherapy** is clearly of benefit in postmenopausal cancer but not in premenopausal cancer.

Renal / Urinary Pathology

The kidney is a complex organ often affected by a variety of acquired and congenital disorders. Common disorders include glomerulonephritis and neoplasms such as Wilms' tumor and renal cell carcinoma. Because the kidney plays such a central role in homeostasis, damage to the organ can have far-reaching effects. For example, since the kidney is involved in blood pressure regulation through the renin-angiotensin system and in the regulation of red blood cell (RBC) production through its synthesis of erythropoietin, renal disease can cause both hypertension and anemia.

The kidney is also frequently affected by systemic disease, such as autoimmune disorders, amyloidosis, septicemia, and diabetes. In fact, renal failure is one of the most common causes of death in systemic lupus erythematosus and diabetes.

This chapter will focus on the disease processes associated with the kidney as well as disorders of the rest of the urinary system.

CONGENITAL ANOMALIES OF THE KIDNEY

A. **Agenesis.** Bilateral agenesis is incompatible with life. Unilateral agenesis may have adequate renal function but may develop progressive glomerular sclerosis.

B. **Hypoplasia** is the failure of the kidneys to develop to normal weight; it is usually unilateral. There are a decreased number of calyces and lobules.

C. **Horseshoe kidney** is a fusion of the kidneys, usually at the lower pole. It is found in 1 in 750 autopsies. Patients have normal renal function but may be predisposed to renal calculi.

D. **Abnormal locations.** The most common abnormal location is the pelvic kidney. There is normal function; however, tortuosity of ureters may predispose to pyelonephritis.

CYSTIC DISEASE

A. **Childhood polycystic disease**

1. **Incidence.** This is a rare autosomal recessive disease.
2. **Clinical features.** Patients present in infancy with progressive renal failure; if the infant survives, he may develop hepatic fibrosis and portal hypertension.
3. **Pathology**
   a. **Grossly,** there are bilaterally enlarged kidneys with smooth surfaces. The cut section shows a sponge-like appearance, with multiple small cysts in the cortex and medulla (Figure 9-1).
   b. **Microscopically,** there is a cylindrical dilatation of tubules.
   c. Most cases also have multiple hepatic cysts.

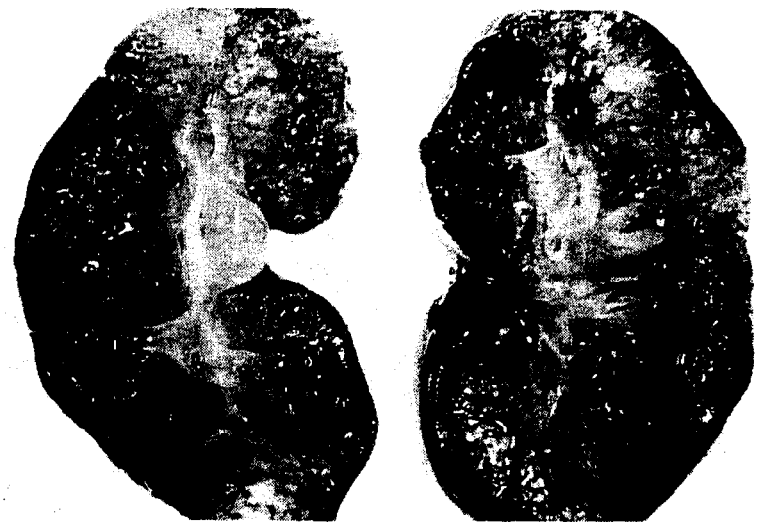


Figure 9-1. Infantile polycystic kidneys (gross).

B. **Adult polycystic disease**

1. **Incidence.** This disease affects 1 in 500 people, showing **autosomal dominant** inheritance. It has a negative family history in 25% of cases, implying a new mutation.
2. **Clinical features.** Patients usually have normal renal function until middle age, at which time they present with renal insufficiency, hematuria, flank pain, and hypertension. Extrarenal manifestations include liver cysts, berry aneurysms in the circle

of Willis, mitral valve disease, and colonic diverticula. Most patients develop hypertension, and 50%-75% develop end-stage renal failure by their seventh decade.

3. **Pathology**
   a. **Grossly,** there is marked bilateral enlargement with large cysts bulging through the surface.
   b. **Microscopically,** functioning nephrons are present between the cysts. Cysts involve less than 10% of nephrons, but they gradually expand and compress the rest of the kidney, interfering with its function. This is the reason why kidney function can remain normal for many years.

Figure 9-2. Polycystic right kidney in an adult (gross).

NOTE

Adult polycystic disease → think liver cysts and berry aneurysms in addition to renal cysts.

HYPERTENSION

Hypertension is defined as an elevated blood pressure greater than 140/90. Primary (essential) hypertension has an unknown etiology and represents 90% of cases. Secondary hypertension makes up the remaining 10% of cases and may be secondary to renal, vascular, endocrine, or neurogenic disorders.

A. **Essential hypertension**

1. **Etiologies**
   a. **Environmental factors.** High dietary sodium in predisposed patients exacerbates the problem. Obesity, stress, and oral contraceptives may contribute to the development of hypertension.
   b. **Physiologic theories**

NOTE

Factors that may raise blood pressure:

- ↑ *Glucocorticoids, T_3, T_4*
- ↑ *Norepinephrine, epinephrine, growth hormone*
- ↑ *Aldosterone*

(1) A defect in sodium excretion would raise blood pressure by a loss of autoregulatory function (i.e., loss of ability to respond to elevated blood pressure by excreting excess sodium and water).

(2) An increase in peripheral resistance could come from increased sympathetic tone.

2. **Pathology.** There is frequently a hyaline deposition in arteriolar walls, narrowing the lumen. High blood pressure may cause atrophy and scarring of the glomeruli and tubules.

B. **Secondary hypertension**

1. **Etiologies**
   a. **Renal disease.** Chronic renal disease, acute glomerulonephritis, and renin-producing tumors all lead to high blood pressure.
   b. **Vascular disease.** Coarctation of the aorta and renal artery stenosis both reduce renal blood flow, leading to increased renin production and hypertension.
2. **Pathogenesis of renal hypertension**
   a. **Increased renin secretion**
      (1) Renin, which is released from the juxtaglomerular apparatus, converts angiotensinogen to angiotensin I, which is converted to angiotensin II in the lung. Angiotensin II causes arteriolar constriction and stimulates aldosterone secretion by the adrenal cortex. Aldosterone causes sodium retention, which leads to an increased intravascular volume.
      (2) Malignant hypertension, unilateral renal artery stenosis, renin-producing tumors, vasculitis, and chronic renal failure all lead to increased renin production.
   b. **Decreased renal antihypertensive substances.** Prostaglandins and kinins typically lower blood pressure; however, they cannot be synthesized normally in renal failure.
   c. **Renal artery stenosis** is a potentially curable form of hypertension. It stimulates renin secretion because of reduced blood flow past the juxtaglomerular apparatus.
      (1) **Pathology.** It is most commonly caused by atherosclerosis (in patients > 50 years) or fibromuscular dysplasia (in patients < 20 years). The kidney exhibits ischemic atrophy with interstitial atrophy and a chronic inflammatory infiltrate.
      (2) **Diagnosis** is made by **arteriography.** Not all anatomic lesions have functional significance.

C. **Malignant hypertension** is a syndrome of severe hypertension (blood pressure is usually > 200/140, but there is no absolute limit) and acute end-organ damage. It usually occurs in patients with long-standing, poorly controlled hypertension. The 5-year mortality rate is 60%-70%.

1. **Clinical features**
   a. There may be manifestations of **increased intracranial pressure**, including papilledema (with retinal hemorrhages and exudates), headache, vomiting, and scotomas. Symptoms may progress to loss of consciousness and seizures and may also cause subarachnoid or intracerebral bleeds.
   b. **Cardiac failure.** Left ventricular dysfunction may occur early.
   c. **Malignant nephrosclerosis** may lead to proteinuria, hematuria, and sometimes acute renal failure. Patients with renal failure have a higher mortality rate.

GLOMERULAR DISEASES

A. **Overview**

1. **Glomerular response** to damage can take several forms.
   a. **Cellular proliferation** may include mesangial, epithelial, and endothelial cells.
   b. **Thickening of the basement membrane** of the glomerular capillaries most often results from subepithelial deposition of fibrin and immune complexes, followed by secretion of more basement membrane material by endothelial and epithelial cells.
   c. **Leukocytic infiltration.** Neutrophils and monocytes may be attracted by antigen-antibody (Ag–Ab) complexes.
   d. **Sclerosis and hyalinization** are due to an accumulation of eosinophilic material, composed of plasma proteins and mesangial matrix. This may lead to irreversible injury.

2. **Pathogenesis**
   a. **Anti-glomerular basement membrane antibodies.** Nephritis results from antibodies against fixed antigens in the glomerular basement membrane, and is the basic mechanism of **Goodpasture's syndrome,** which also includes antibodies against the basement membrane in pulmonary alveoli.
   b. **Antibodies against other antigens.** Glomerulonephritis may result from antibodies against other fixed antigens or antibodies in glomeruli.

NOTE

Goodpasture's: *anti-glomerular basement membrane and anti-alveolar basement membrane.*

Goodpasture's is also discussed in the Respiratory Pathology chapter.

c. **Circulating immune complexes.** Ag–Ab complexes become trapped within glomeruli, causing glomerular injury. Antigens may be exogenous (e.g., serum sickness) or endogenous (e.g., systemic lupus erythematosus with DNA–anti-DNA complexes).

d. **Mediators of injury.** After Ag–Ab interaction, injury may result from a variety of mechanisms, including activation of the complement system, macrophages, and the coagulation system, and attraction of neutrophils and monocytes.

B. **Clinical syndromes in glomerular disease**

1. **Nephritic syndrome.** Patients with acute nephritis present with **proteinuria, hematuria, red blood cell (RBC) casts,** and varying degrees of renal insufficiency and hypertension.

2. **Nephrotic syndrome** is a clinical tetrad of generalized **edema, severe proteinuria, hypoalbuminemia,** and **hyperlipidemia.** It results from a loss of the charge barrier of the glomerular basement membrane (GBM) with an increased permeability to albumin. This leads to massive proteinuria and edema. The most common cause of nephrotic syndrome in children is **lipoid nephrosis (minimal change disease)**; in adults, the most common cause is **membranous glomerulonephritis.**

3. **Rapidly progressive glomerulonephritis (RPGN).** Also called **"crescentic GN,"** RPGN is a syndrome of rapidly deteriorating renal function that accompanies glomerular injury. Clinically, patients present with a nephritic urine sediment and renal failure.

C. **Glomerulonephritis**

1. **Acute poststreptococcal glomerulonephritis**

a. **Clinical features.** This disease affects children more frequently than adults and usually occurs 1–2 weeks after streptococcal infection of the throat or skin. Laboratory studies show **elevated antistreptolysin O** (ASLO) titers and **low serum complement.**

b. **Pathogenesis.** The mechanism is immune-related; the disease is probably due to immune complex desposition.

2. **Lipoid nephrosis (minimal change disease)**

a. **Clinical features.** This is the most common cause of nephrotic syndrome in children. Its peak incidence is 2-3 years of age, and it may be associated with food allergy, certain medications, or hematologic malignancies.

b. **Prognosis.** Renal function does not usually deteriorate. This syndrome is usually steroid-responsive, especially in children. Complete recovery is expected.

3. **Membranous glomerulonephritis**
   a. **Clinical features.** This is the most common cause of nephrotic syndrome in adults, but it is rare in children. There is usually an insidious onset of proteinuria; hematuria and mild hypertension may occur. There may be a genetic predisposition. Most cases are idiopathic, but some are associated with infection, drugs, tumors, and systemic disease.

4. **Membranoproliferative glomerulonephritis (MPGN)**
   a. **Clinical features.** Two-thirds of patients have the nephrotic syndrome; the rest have non-nephrotic range proteinuria or a mixed nephritic/nephrotic picture. MPGN accounts for 5%–10% of cases of idiopathic nephrotic syndrome in adults and children. MPGN may be secondary to many systemic disorders, including complement deficiency, chronic infections, and chronic lymphocytic leukemia.
   b. **Prognosis** is poor, and treatment is controversial. The disease is slowly progressive. Half of all patients die of chronic renal disease within 10 years of the diagnosis.

5. **Focal segmental glomerulosclerosis**
   a. **Clinical features.** It accounts for 10%–15% of cases of nephrotic syndrome in children and adults. As compared to lipoid nephrosis, these patients more often have hematuria, hypertension, impaired GFR, and nonselective proteinuria.
   b. **Pathogenesis.** The mechanism is probably immunologic (possibly an aggressive variant of lipoid nephrosis) or a secondary reaction of residual nephrosis to nephron loss. Intravenous drug abuse is implicated in some patients.
   c. **Prognosis** is poor; some patients, especially children, respond to steroids. More than 50% of patients develop end-stage renal disease within 10 years of diagnosis. There is a high rate of recurrence in transplants.

6. **Anti-GBM antibody disease**
   a. **Clinical features.** This disease causes **rapidly progressive glomerulonephritis (RPGN).** When accompanied by pulmonary involvement, it is known as **Goodpasture's syndrome.**
   b. **Pathogenesis.** The mechanism involves antibodies directed against a collagen component of basement membranes.

NOTE

- *"Focal" refers to involvement of only some glomeruli.*
- *"Diffuse" means all glomeruli are involved.*
- *"Segmental" means only parts of the glomerulus are involved.*
- *"Global" means the entire glomerulus is involved.*

NOTE

Plasmapheresis involves filtering of plasma to remove Ag–Ab complexes.

7. **Chronic glomerulonephritis**
   a. **Clinical features.** This is the final stage of many forms of glomerular disease, so the rate of development is variable. Patients may present with anemia, anorexia, malaise, nausea, vomiting, proteinuria, hypertension, and azotemia.
   b. **Pathogenesis.** The mechanism depends on the underlying etiology. It may follow RPGN, membranous glomerulonephritis, MPGN, IgA nephropathy, focal segmental glomerulosclerosis, and others. It is rare after poststreptococcal glomerulonephritis. Twenty-five percent of patients with chronic glomerulonephritis have no documented history of acute glomerulonephritis.
   c. **Prognosis** is poor. Patients usually progress to end-stage renal disease.
   d. **Pathology**
      (1) **Grossly,** shrunken kidneys are seen.

D. **Hereditary nephritis (Alport's syndrome)** is a hereditary abnormality of collagen, resulting in renal disease, deafness, and ocular abnormalities (e.g., dislocated lens, corneal dystrophy, cataracts).

1. **Incidence.** It is primarily an X-linked disorder; women are carriers with mild forms and men develop the full-blown syndrome.
2. **Clinical features.** Patients have hematuria and proteinuria, which slowly progress to renal failure.

E. **Glomerular injury in systemic disease**

1. Systematic lupus erythematosus
2. Amyloidosis
3. Diabetes mellitus
4. Goodpasture's syndrome
5. Wegener's granulomatosis
6. **Bacterial endocarditis** may lead to **immune complex nephritis.** It produces focal, segmental necrotizing glomerulonephritis, MPGN, or rapidly progressive glomerulonephritis with crescent formation. It is associated with low serum complement levels and usually reverses with treatment of the infection.
7. **Multiple myeloma** is a hematologic malignancy characterized by **overproduction of monoclonal immunoglobulins** and often excess monoclonal light chains. The kidney in multiple myeloma can show a variety of pathologic lesions, including **tubular plugging by casts of myeloma protein (myeloma kidney),** amyloid, hypercalcemic nephropathy, and light-chain deposition disease.

ACUTE TUBULAR NECROSIS (ATN)

ATN is acute renal failure associated with reversible injury to the tubular epithelium. It is the most common cause of acute renal failure (Figure 9-3).

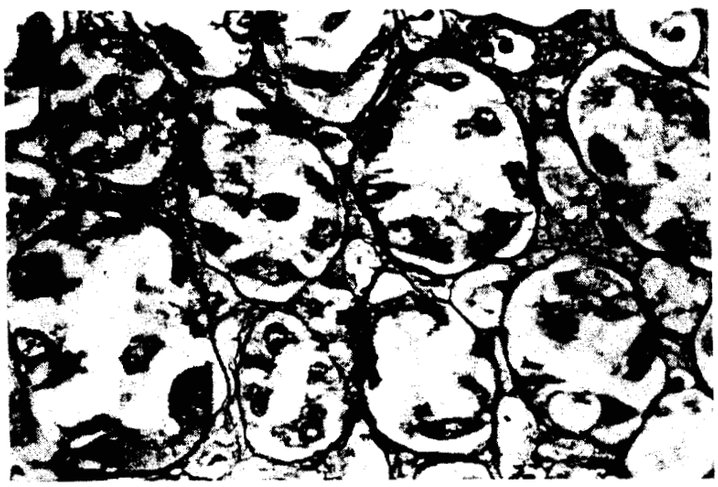


Figure 9-3. Acute tubular necrosis.

A. **Types**

1. **Ischemic ATN** is due to decreased blood flow caused by severe renal vasoconstriction, hypotension, or shock. It is the most common cause of ATN.
2. **Nephrotoxic ATN** is caused by heavy metals (e.g., mercury, lead, gold), drugs (e.g., polymyxin, methicillin, sulfonamides), organic solvents (e.g., carbon tetrachloride, chloroform, methyl alcohol), ethylene glycol, phenol, pesticides, or myoglobin.

B. **Pathogenesis.** Ischemia or toxins cause tubular damage and may lead to:

1. **Vasoconstriction** of preglomerular arterioles, leading to a decreased GFR
2. **Tubular obstruction** by casts formed from tubular debris. (Urinary obstruction causes increased intraluminal pressure and decreased GFR.)
3. **Back leakage of fluid** from the tubules into the interstitium, causing increased interstitial pressure and tubular collapse
4. **Decreased glomerular** capillary permeability

C. **Clinical features.** ATN has four phases:

1. In the **initial phase**, the precipating event (e.g., shock, toxins) occurs.
2. During the **oliguric phase**, there is decreased urine output. Uremia, fluid overload, and hyperkalemia may occur.
3. During the **diuretic phase**, there is a gradual increase in urine volume. Hypokalemia, electrolyte imbalances, and infection may occur.
4. In the **recovery phase**, there is an improved concentrating ability, normalization of blood urea nitrogen (BUN) and creatinine, and restoration of tubular function as new epithelial cells grow in.

D. **Prognosis** is excellent if the patient survives the disease responsible for the ATN.

TUBULOINTERSTITIAL DISEASE

A. **Pyelonephritis** is an infection of the renal pelvis, tubules, and interstitium, i.e., everything but the glomerulus.

1. **Etiology.** Etiologic agents are usually Gram-negative bacilli (e.g., *Escherichia coli,* Proteus, Klebsiella, Enterobacter), or *S. faecalis.* In general, etiologic agents are organisms derived from the patient's fecal flora.
2. **Pathogenesis**
   a. **Ascending infection** is the most common route. The sequence of events is as follows:
      (1) First, there is colonization of the distal urethra and vaginal introitus by bacteria.
      (2) Bacteria enter the bladder, facilitated by urethral instrumentation, short urethras, or urethral trauma during intercourse.
      (3) There is an inability to clear urine from the bladder. Urinary stasis is caused by bladder obstruction or inability to fully empty the bladder as seen during pregnancy, bladder diverticula, or benign prostatic hypertrophy.
      (4) Proliferation of bacteria in the urine leads to **cystitis,** infection of the urinary bladder, causing frequency, urgency, dysuria, and suprapubic pain. Systemic signs (e.g., fever) are uncommon.
      (5) **Vesicoureteral reflux (VUR)** allows bacteria to ascend to the kidneys; during micturition, urine is forced up one or both ureters.

NOTE

Tubulointerstitial disease usually affects females more than males because the female urethra is shorter.

(6) Intrarenal reflux permits spread of bacteria to the renal parenchyma.

b. **Hematogenous infection** is much less common as a source of pyelonephritis.

3. **Acute pyelonephritis**
   a. **Pathogenesis.** Predisposing factors are urinary obstruction, vesicoureteral reflux, pregnancy, urethral instrumentation, diabetes mellitus, and other renal pathology.
   b. **Incidence.** Women predominate among patients under age 40. In later years, there is an increasing incidence in men due to benign prostatic hypertrophy.
   c. **Clinical features** include fever, malaise, dysuria, frequency, urgency, and **costovertebral angle tenderness.** Urine shows many WBCs and WBC casts. Urine culture typically shows greater than one million organisms per milliliter.

4. **Chronic pyelonephritis** is characterized by interstitial parenchymal scarring, which involves and deforms the calyces and pelvis.
   a. **Pathogenesis**
      (1) **Reflux nephropathy** is the most common type. It results from VUR and subsequent infection.
      (2) **Chronic obstructive nephropathy** results from infection superimposed on urinary obstruction.
   b. **Clinical features.** There may be an insidious or acute onset. Patients present with renal failure and hypertension. Pyelograms are diagnostic. Proteinuria is a poor prognostic sign.

5. **Toxic nephritis**
   a. **Acute allergic interstitial nephritis** is a hypersensitivity reaction to infection or drugs (e.g., NSAIDs, synthetic penicillins, sulfonamides, furosemide, rifampin), resulting in interstitial edema with a mononuclear infiltrate.
   b. **Analgesic nephritis** is interstitial nephritis and renal papillary necrosis, induced by large doses of analgesic combinations (usually phenacetin and aspirin).

6. **Other forms of tubulointerstitial nephritis**
   a. **Gouty nephropathy** is the deposition of urate crystals in tubules.
   b. **Acute urate nephropathy** is due to precipitation of crystals in the collecting ducts, causing obstruction.
   c. **Hypercalcemia** results in calcium deposition in the kidney and stone formation.
   d. **Multiple myeloma.** Some **Bence-Jones proteins** are directly toxic to tubular epithelium and also lead to cast formation and urinary obstruction.

NOTE

Anytime there is fluid stasis, bacteria can multiply.

VASCULAR AND ISCHEMIC DISEASE

A. **Renal infarcts**

1. **Pathogenesis.** Ischemia may be caused by embolization of mural thrombi or valvular vegetations of the left heart, aortic dissection, or paradoxical embolization from aortic aneurysm.
2. **Pathology.** There are sharply demarcated pale regions, usually wedge-shaped, that undergo necrosis with subsequent scarring.
3. **Clinical features.** Infarcts may be asymptomatic, or they may cause pain, hematuria, and hypertension.

B. **Diffuse cortical necrosis**

1. **Pathogenesis.** Disseminated intravascular coagulation (DIC) or vasoconstriction can lead to ischemia of the entire cortex.
2. **Pathology.** Ischemic necrosis of the renal cortex may develop.
3. **Clinical features** are acute anuria and uremia.

C. **Renal vein thrombosis**

1. **Pathogenesis.** Thrombosis of one or both renal veins may occur. This condition is associated with the nephrotic syndrome, particularly membranous glomerulonephritis, although a causal relationship is not established. Renal cell carcinoma may also provoke vein thrombosis as a result of direct invasion by tumor.
2. **Clinical features.** Thrombosis may present with hematuria, flank pain, and renal failure.
3. **Pathology**
   a. **Grossly,** the kidney is enlarged, and the vein contains a thrombus.

D. **Sickle cell anemia.** Blood in the vasa recta tends to sickle in response to the hypertonic, hypoxic milieu of the renal medulla. This produces patchy papillary necrosis and occasional cortical scarring.

UROLITHIASIS

A. **Incidence.** Urolithiasis occurs in up to 6% of the population; men are affected more often than women.

B. **Pathogenesis.** There is a familial predisposition, which depends on the type of stone.

Figure 9-4. Nephrolithiasis (gross).

1. **Calcium-containing stones** (75%–80%). Most patients have hypercalciuria without hypercalcemia; 20% have hyperuricosuria.
2. **Magnesium-ammonium phosphate ("struvite") stones** (15%) occur after infection by **urea-splitting bacteria** (such as Proteus), which transform urea into ammonia.
3. **Uric acid stones** (5%) are seen in gout, leukemia, and in patients with acidic urine.
4. **Cystine stones** (1%) are associated with an inborn error of metabolism (e.g., cystinuria, an autosomal recessive amino acid transport disorder). They are very rare.

C. **Pathology.** Most stones are unilateral and are formed in the calyx, pelvis, and bladder (Figure 9-5).

D. **Clinical features.** Calcium stones are radiopaque; they are the only ones that can be seen on x-ray.

OBSTRUCTIVE UROPATHY AND HYDRONEPHROSIS

A. **Etiologies** include urolithiasis, benign prostatic hypertrophy, pregnancy, neurogenic bladder, tumor, inflammation, and congenital anomalies (e.g., posterior urethral valves, strictures).

B. **Pathogenesis. Hydronephrosis** is the persistence of glomerular filtration despite urinary obstruction, causing dilation of calyces and pelvis and reabsorption of the filtrate into the vascular sys-

BRIDGE TO MICROBIOLOGY

When you see Proteus, think: urea-splitting, stone-forming, and alkaline urine.

Figure 9-5. Hydronephrosis, gross.

tem. A high pressure in the collecting system causes atrophy and ischemia.

C. **Pathology.** There is dilatation of the pelvis and calyces with blunting of renal pyramids, leading to progressive parenchymal atrophy (Figure 9-5).

D. **Clinical features**

1. **Unilateral hydronephrosis** may remain asymptomatic as the kidney atrophies.
2. **Bilateral, incomplete hydronephrosis** causes the patient to lose concentrating ability, causing urinary frequency, polyuria, nocturia, and hypertension.
3. **Bilateral, complete hydronephrosis** causes anuria, uremia, and death if untreated.

TUMORS OF THE KIDNEY

A. **Benign tumors**

1. **Cortical adenomas** are a common finding at autopsy. Histologically, they may be identical to renal cell carcinoma and are distinguished by size (adenomas are less than 3 cm).
2. **Angiomyolipomas** are hamartomas, composed of fat, smooth muscle, and blood vessels.
3. **Renal fibroma (hamartoma)** is an incidental finding at autopsy. They are small grey nodules within the pyramids.

Figure 9-6. Renal cell carcinoma, gross.

B. **Malignant tumors**

1. **Renal cell carcinomas** are adenocarcinomas, arising from the proximal convoluted tubule.
   a. **Incidence.** They form 90% of all renal cancers in adults. Men and women have about equal incidence. They are most common from age 50-70.
   b. **Pathogenesis.** There is a moderate association with smoking and a familial predisposition.
   c. **Pathology.** Histology may be identical to adenoma, but tumors are prone to metastases if larger than 3 cm.
      (1) **Grossly,** tumors are 3-15 cm yellow lesions found most commonly in the upper pole; they are usually solitary. Commonly, there are areas of necrosis and hemorrhage. The tumor often invades the renal vein and extends into the vena cava and heart.
   d. **Clinical features**
      (1) There is a "classic" triad of hematuria, palpable mass, and costovertebral pain that occurs in only 10% of cases; hematuria is most important.
      (2) Renal cell carcinomas may remain asymptomatic until they are very advanced. They also may cause paraneoplastic syndromes from ectopic hormone production: polycythemia (erythropoietin production), hypertension (renin production), Cushing's syndrome (corticosteroid synthesis), hypercalcemia (PTH-like hormone), and feminization or masculinization (gonadotropin release).
      (3) They also may cause amyloidosis, a leukemoid reaction, or eosinophilia.

e. **Metastases.** There is a high incidence of metastasis on initial presentation. Sites include lungs, bones, lymph nodes, liver, adrenals, brain, and the opposite kidney. Metastases are mainly hematogenous and lymphatic.

f. **Prognosis.** Five-year survival depends on stage, but it is especially poor (25%-50%) if the tumor extends into the renal vein.

2. **Wilms' tumor (nephroblastoma)** is a tumor derived from mesonephric mesoderm and composed of epithelium, bone, cartilage, and muscle.

a. **Incidence.** This is a rather common childhood malignancy with peak incidence at age 2.

b. **Clinical features.** Patients present with an abdominal mass as well as hypertension, nausea, hematuria, or intestinal obstruction.

c. **Pathology**

(1) **Grossly,** most tumors are unilateral but may be bilateral if familial.

(2) Tumor cells contain microdeletions in chromosomes. The gene has been localized to chromosome 11p.

d. **Metastases.** Areas include lymph nodes, lungs, liver, and adrenals.

e. **Prognosis.** There is a 90% survival rate when patients are treated with surgery, chemotherapy, and radiotherapy.

3. **Carcinomas of renal pelvis**

a. **Incidence.** These make up 5–10% of primary renal tumors.

b. **Clinical features.** They usually present early with hematuria, and they may cause hydronephrosis and flank pain.

URETERS

A. **Congenital anomalies**

1. **Double ureters** form when ureters join at some point before the junction to the bladder ("Y"-shaped) or enter the bladder separately. This anomaly is associated with double renal pelves or an abnormally large kidney.

2. **Aberrant renal vessel.** Usually, an aberrant artery arises from a renal artery of the aorta and supplies the lower pole. It may cause ureteropelvic obstruction.

B. **Ureteritis** is an inflammation of the ureter, usually as a result of urinary tract infections.

C. **Ureteral obstruction** results in hydroureter and hydronephrosis.

1. **Internal obstruction**
   a. **Renal calculi** are the most common cause.
   b. **Strictures** may be congenital or acquired (e.g., postsurgical, inflammatory).
   c. **Hematomas** may result from bleeding in the kidney or proximal ureter.
   d. **Tumors** form intraluminal masses and thickening of the ureteral wall.
2. **External obstruction**
   a. **Inflammation** leads to scar formation.
   b. Pelvic **tumors** may compress or invade the ureteral wall.
   c. **Sclerosing retroperitonitis** is a fibrosis of retroperitoneal structures, possibly caused by an autoimmune mechanism.
   d. Pregnancy does not cause obstruction, but it does cause dilation of the ureters by an unknown mechanism.

D. **Tumors.** Primary tumors are rare. Benign tumors rarely cause obstruction; malignant tumors are usually associated with bladder carcinomas.

BLADDER

A. **Congenital anomalies**

1. **Diverticula** are pouch-like evaginations of the bladder wall. They occur in older men and women. They may lead to urinary stasis and infection.
   a. **Congenital** diverticula are due to abnormal development of musculature and are usually single.
   b. **Acquired diverticula** result from obstruction of the urethra or bladder neck.
2. **Exstrophy of bladder** is due to the absence of the anterior musculature of the bladder and abdominal wall as a result of the failure of down-growth of mesoderm over the anterior bladder. It is usually the site of severe chronic infections, and it leads to an increased incidence of adenocarcinoma.
3. **Patent urachus** is a fistula that connects the bladder with the umbilicus.
4. **Urachal cysts** are due to the persistence of the central urachus. Carcinomas may develop in these cysts.

B. **Cystitis**

1. **Etiology.** Organisms responsible are usually the patient's fecal flora (e.g., *Escherichia coli,* Proteus, Klebsiella, Enterobacter, *Streptococcus faecalis,* or Staphylococcus).
2. **Clinical features.** Cystitis causes frequency, urgency, dysuria, and suprapubic pain. Systemic signs (e.g., fever, malaise, chills) are uncommon with lower urinary tract infections.

C. **Bladder obstruction**

1. **Etiology**
   a. In **men**, prostatic enlargement as a result of benign hyperplasia or carcinoma is the most common cause.
   b. In **women**, cystocele of the bladder is the most common cause.
2. **Pathology.** Thickening and hypertrophy of the smooth muscle of the bladder wall leads to trabeculation. This may cause the development of diverticula.

D. **Miscellaneous lesions**

1. **Fistulas.** The most common fistula is a vesicovaginal fistula, which often results from irradiation or malignancy.
2. **Calculi** are usually asymptomatic, though they may cause inflammation of the bladder wall. They may form in the bladder or more proximally in the genitourinary tract.

E. **Tumors.** Ninety percent of primary bladder neoplasms are derived from transitional bladder epithelium called **urothelium**.

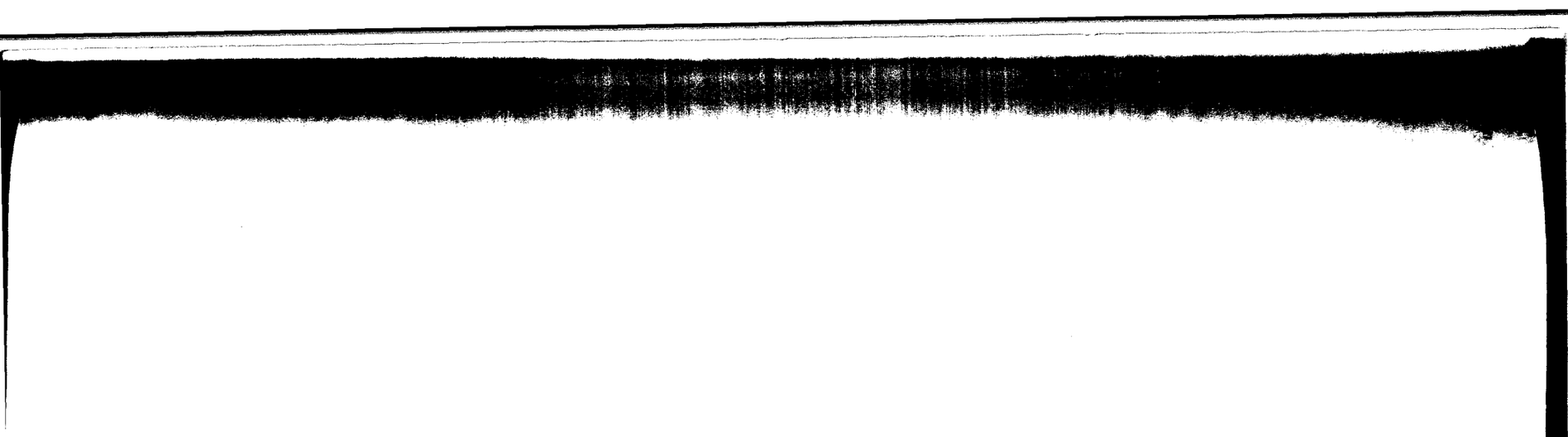
1. **Papillomas** are uncommon and may present with hematuria.
2. **Carcinomas**
   a. **Etiology.** Risk factors include:
      (1) Exposure to industrial chemical compounds (the risk may be increased 50 times with prolonged exposure)
      (2) Infection with ***Schistosoma haematobium***
      (3) Cigarette smoking
   b. **Types. Transitional cell carcinoma** of the urothelium forms 90% of cases. Other forms, including squamous cell carcinomas and adenocarcinomas, are rare.
   c. **Incidence.** Urothelial cell cancer causes 3% of all cancer deaths in the United States in both men and women. The peak incidence is between 40 and 60 years of age.
   d. **Clinical features.** Bladder cancer usually presents with painless hematuria. It may also cause dysuria, urgency, frequency, hydronephrosis, and pyelonephritis.
   e. **Prognosis.** Bladder cancer has a high incidence of recurrence. The prognosis depends on grade and stage; overall 5-year survival is 30%.
3. **Sarcomas** are large polypoid masses that protrude into the vesical lumen. They are usually leiomyosarcomas.

URETHRA

A. **Urethritis** presents with itching, pain, and urinary frequency. It may be **gonococcal or nongonococcal.** Organisms responsible for nongonococcal urethritis include Chlamydia, Mycoplasma, and enteric bacteria. **Reiter's syndrome** is the triad of urethritis, arthritis, and conjunctivitis.

B. **Tumors**

1. **Caruncles** are red, small, painful benign masses in the external urethral meatus of affected women.
2. **Carcinomas** are rare. They occur more frequently in the elderly, arising at the external meatus. They are wart-like, papillary growths, composed of malignant squamous cells, which may protrude into the lumen. They are often caused by papillomaviruses and are increasing in incidence among younger age groups as sexually transmitted diseases in immunocompromised hosts (i.e., patients with AIDS).



Hematologic / Lymphoreticular Pathology

Disorders of the heme/lymph system can result from underproduction or overproduction of the formed elements of the blood, hematologic malignancies, or autoimmune disorders. This chapter will discuss the different types of disorders, their clinical presentations, and their distinguishing features. Disorders involving the spleen and thymus will also be reviewed.

ANEMIAS

Anemias are a group of disorders characterized by a decrease in the number of circulating erythrocytes. This decrease is reflected in the laboratory values of hemoglobin and hematocrit (the percentage of blood volume composed of RBCs).

A. **Signs and symptoms**

1. Anemia causes palpitations, systolic cardiac murmurs, high-output heart failure, pallor, orthostatic hypotension (in cases of decreased blood volume), fatigue, dizziness, syncope, and angina (due to impaired oxygen transport).
2. Anemia can also be caused by an underlying disease. Examples are infection, bleeding as a result of leukemia, or tissue infarction due to sickle cell crisis.

B. **Classification**

1. **RBC morphology.** RBCs may be normal in size **(normocytic),** large **(macrocytic),** or small **(microcytic).** RBC size is measured in terms of the **mean corpuscular volume** (MCV). Hgb content may be normal **(normochromic)** or decreased **(hypochromic).**
2. **Pathogenesis.** Anemia may result from blood loss, increased destruction of RBCs (hemolysis), or decreased production of

RBCs. The **reticulocyte count** measures the percent of circulating RBCs that are newly synthesized reticulocytes.

a. Patients suffering from a hypoproliferative anemia have low reticulocyte counts.
b. Patients with blood loss or hemolysis should have a compensatory increase in the reticulocyte count, assuming the marrow is normal.

C. **Anemia secondary to loss or destruction of RBCs**

1. **Blood loss**
   a. **Clinical features.** There are variable presentations, depending on rate and severity of blood loss. Chronic blood loss is generally better tolerated, as the bone marrow is able to regenerate lost RBCs by increasing erythropoiesis. Acute blood loss is dangerous; hypovolemia may lead to shock and death unless intravascular fluid deficits are rapidly restored.
   b. **Pathology.** The peripheral blood smear initially shows normocytic normochromic RBCs. As the marrow begins to produce more RBCs, the reticulocyte count increases; these cells are macrocytic and polychromatophilic. Mobilization of platelets and leukocytes may lead to thrombocytosis and leukocytosis.
2. **Hemolytic anemias** are a group of disorders characterized by **premature RBC destruction,** hemoglobin (Hb) breakdown, and a compensatory increase in erythropoiesis. Hemolysis can be **intravascular** with elevated serum and urinary Hb, jaundice, urinary hemosiderin, and decreased circulating haptoglobin. **Extravascular** hemolysis generally occurs within organs of the reticuloendothelial system. Some hemolytic anemias occur on the basis of a defect inherent to the RBC (intracorpuscular mechanism); others are due to factors extrinsic to the RBC, such as antibodies (extracorpuscular mechanism). The compensatory acceleration in erythropoiesis may lead to intramedullary erythroid hyperplasia, extramedullary erythropoiesis, skeletal deformities, bile pigment gallstones, and the presence of nucleated RBCs in the peripheral blood.
   a. **Hereditary spherocytosis**
      (1) **Clinical features.** There is an autosomal dominant, intrinsic defect in erythrocyte membrane **spectrin** molecules, leading to a less pliable, spherical RBC vulnerable to destruction in the spleen. The disease may present with anemia, jaundice, splenomegaly, cholelithiasis, or all four.

NOTE

Use the reticulocyte count to evaluate RBC production:

↑ reticulocyte count = ↑ production

When chronic blood loss leads to iron deficiency, the reticulocyte count will be low because iron is necessary to make reticulocytes.

CLINICAL CORRELATE

Hematocrit is not a good indicator of anemia in the setting of acute blood loss—the hematocrit won't fall until the extravascular fluid has had time to re-equilibrate with blood to restore intravascular volume.

NOTE

The effects of compensatory mechanisms depend on whether RBC destruction is acute or chronic. For example, bile pigment gallstones arise in hereditary spherocytosis but not in an acute episode of hemolysis due to G6PD deficiency.

b. **G6PD deficiency**

(1) **Clinical features.** The disease is due to an X-linked deficiency of the first enzyme in the hexose monophosphate shunt. RBCs defend themselves against oxidative injury via glutathione, which is maintained in a reduced form by NADPH. G6PD is necessary to generate NADPH. In the absence of G6PD, oxidative stress on the erythrocyte leads to hemolysis.

c. **Sickle cell disease**

(1) **Clinical features.** A hereditary hemoglobinopathy is present in 2% of the African-American population of the U.S. (approximately 8% carry the trait). HbS has a **substitution of valine for glutamic acid** at position 6 of the beta chain of Hb. The HbS molecules aggregate and polymerize when deoxygenated; this leads to a change in RBC shape called "sickling," with resultant **microvascular occlusion and hemolysis.** Although initially reversible, the sickling process eventually leads to irreversible RBC membrane changes. The tendency of RBCs to sickle depends on the concentration of HbS: heterozygotes who have approximately 60% HbA and 40% HbS ("sickle trait") sickle only in extreme conditions; homozygotes for HgbS ("sickle cell anemia") sickle under less extreme conditions. Factors that predispose to sickling include hypoxia, dehydration, and low pH.

(2) **Course.** This is a chronic disease punctuated by acute exacerbations. **Vaso-occlusive crises** ("painful crises") are episodes of hypoxic infarction in bones, lungs, liver, spleen, and other tissues that may be triggered by infection, dehydration, acidosis, or may occur spontaneously. **Aplastic crises** are episodes of inadequate bone marrow activity that may be triggered by infection or folate deficiency. Parvovirus is a common etiologic agent. **Sequestration crises** occur in children or adults who have not yet undergone autosplenectomy, resulting in massive sequestration and splenomegaly with resultant hypovolemia that may lead to shock. In addition to crises, the functional asplenia leaves the patients vulnerable to **Salmonella osteomyelitis,** and to infections with **encapsulated organisms** such as Pneumococcus. Most patients die prior to age 30.

d. **Thalassemias.** The thalassemias are an inherited group of disorders characterized by absent or decreased synthesis of either the alpha or the beta chain of Hb.

FLASHBACK TO BIOCHEMISTRY

Now is a good time to review the hexose monophosphate shunt. It is discussed in the Carbohydrates chapter of the Biochemistry review book.

IN A NUTSHELL

α-thalassemia is due to gene deletion. β-thalassemia is due to defects in mRNA processing.

α -thalassemia:

# of genes deleted	***type***
1	*Silent carrier*
2	*α-thal trait*
3	*HbH disease in adults*
4	*Hydrops fetalis*

(1) **Alpha-thalassemia** (α-thal) is due to decreased or absent synthesis of the alpha chains of Hb. It is of variable clinical severity since the normal person has four alpha genes.

(2) **Beta-thalassemia** (β-thal major and β-thal minor) is due to decreased or absent synthesis of the beta chain, usually as a result of defective mRNA processing. It is most common in the Mediterranean countries, Africa, and Southeast Asia. The result of the defect is a hypochromic cell with a relative excess of alpha chains. These alpha chains aggregate and become insoluble, leading to intra- and extramedullary hemolysis. Severe anemia stimulates erythropoietin secretion, leading to enhanced erythropoiesis in bone, liver, and spleen.

(3) **Pathology**. In **β-thal minor,** the peripheral blood smear shows varying degrees of anisocytosis (variation in size), hypochromia, microcytosis, target cells, and stippled or fragmented RBCs.

(4) **Course.** Alpha-thal may range from asymptomatic (α-thal trait) to lethality in utero (hydrops fetalis). Patients with HbH disease generally have a mild anemia requiring transfusion only at times of accelerated hemolysis or impaired erythropoiesis. Beta-thal major is a severe disease with growth retardation, skeletal deformities, and hepatosplenomegaly. Multiple transfusions may lead to iron overload with resultant systemic effects. Most patients die by the third decade. Beta-thal minor is clinically milder and may be discovered incidentally as a hypochromic, microcytic anemia.

IN A NUTSHELL

Anemias caused by loss or destruction of red blood cells:

- *Blood loss*
- *Hereditary spherocytosis*
- *GGPD deficiency*
- *Sickle cell disease*
- *Thalassemias*

NOTE

The body's vitamin B_{12} stores are so large that it usually takes years to develop a vitamin B_{12} deficiency after absorption stops. Folate deficiency develops much more quickly, on the order of months, in states of vigorous RBC production.

D. **Anemias secondary to decreased erythropoiesis**

1. **Megaloblastic anemias**

a. **Characteristics.** A group of anemias characterized by macro-ovalocytes and large, **hypersegmented neutrophils** (having greater than five lobes per nucleus) in the peripheral blood. Important causes are **vitamin B_{12} and folate deficiency.**

b. **Pathogenesis.** Vitamin B_{12} and folate deficiencies result in impaired DNA synthesis without affecting RNA or protein synthesis; hence, they selectively impair nuclear development. This results in destruction of immature RBCs within the bone marrow (ineffective erythropoiesis), as well as extramedullary hemolysis.

c. **Classification**

(1) **Vitamin B_{12} deficiency** has profound neurologic as well as hematologic sequelae. It may result from multiple

causes, including dietary deficiency, malabsorption, competitive tapeworm uptake, bacterial overgrowth, pregnancy, hyperthyroidism, and cancer. A deficiency of intrinsic factor leads to vitamin B_{12} deficiency and pernicious anemia.

(2) **Folate deficiency** produces megaloblastic anemia without neurologic changes. Folate deficiency may result from deficient intake (poor diet, alcoholism, malabsorption), increased need (pregnancy, malignancy, increased hematopoiesis), or impaired use (antimetabolite drugs).

2. **Iron deficiency anemia**

a. **Clinical features.** Iron deficiency is among the most common nutritional deficiencies worldwide. There are many causes, including poor diet, malabsorption, and pregnancy. By far, the most common cause of iron deficiency anemia is **blood loss,** usually from the gastrointestinal, genitourinary, or female genital tract. The finding of iron deficiency anemia in an elderly patient should alert the clinician to the possibility of an occult source of blood loss, often a malignancy.

b. **Pathology.** The peripheral smear shows **hypochromic, microcytic** changes. The **Plummer-Vinson syndrome** is a triad of microcytic anemia, atrophic glossitis, and esophageal webs.

3. **Aplastic anemia**

a. **Clinical features.** This is a **stem cell defect,** leading to **pancytopenia** (i.e., anemia, neutropenia, thrombocytopenia). There are multiple etiologies: idiopathic, drugs (i.e., alkylating agents, chloramphenicol), radiation, infections, and congenital anomalies (i.e., Fanconi's anemia).

b. **Pathology.** The peripheral smear shows markedly decreased numbers of circulating RBCs, WBCs, and platelets. RBCs are normochromic and normocytic. The **bone marrow is hypocellular** with a few foci of lymphocytes and plasma cells.

c. **Course.** Prognosis is poor. Bone marrow transplant may be curative.

NOTE

The diagnosis of iron deficiency is commonly made by demonstrating low serum iron, high TIBC, and low serum ferritin. There is no stainable iron in the bone marrow. The anemia of chronic disease, also a microcytic anemia, exhibits low serum iron, normal-to-low TIBC, and high ferritin.

POLYCYTHEMIA

Polycythemia is an increase in concentration of circulating erythrocytes. This increase may be primary or secondary, absolute or relative.

A. **Relative polycythemia** occurs due to loss or sequestration of intravascular volume without loss of RBCs. Volume loss may be due to decreased fluid intake, vomiting, diarrhea, burns, or adrenal insufficiency.

B. **Polycythemia vera**

1. **Clinical features.** Polycythemia vera is a **myeloproliferative syndrome** characterized by a marked increase in erythrocyte mass. It is most common in males age 40-60. The etiology is unknown but is probably due to a neoplastic hematopoietic stem cell. There are symptoms of increased blood volume, vascular stasis/thrombosis, or bleeding tendency. Patients may later develop anemia or acute leukemia due to "bone marrow burn out." Folate deficiency may develop due to the hyperproliferative state.
2. **Pathology.** The peripheral smear shows a markedly increased number of RBCs, WBCs, and platelets. Erythropoietin levels are low. The bone marrow shows **erythroid hyperplasia** with excess normoblasts.

C. **Secondary polycythemia**

1. **Clinical features.** An increased RBC mass as a result of **increased erythropoietin levels** is seen. There are multiple etiologies, including high altitude with low O_2, cigarette smoking with high carbon monoxide levels, respiratory disease, cardiac disease (e.g., right-to-left shunts, cardiac failure), hemoglobinopathies, renal disease (e.g., cysts, hydronephrosis), and malignancies (e.g., renal cell carcinoma, hepatoma, leiomyoma, adrenal adenoma, cerebellar hemangioblastoma).
2. **Pathology.** An isolated erythrocythemia without an increase in WBCs or platelets is noted.

THROMBOCYTOPENIA

Thrombocytopenia is a decrease in the platelet count (normal platelet count = 150,000-400,000/mm^3).

A. **Clinical features.** There may be bleeding from small vessels, often skin, gastrointestinal tract, and genitourinary tract. The most common sign is the development of **petechiae** (minute pin-sized hemorrhages in the skin) and **purpura** (large red, nonblanching lesions). Petechiae develop before purpura, which are more often

seen with combined deficiencies of platelets and plasma clotting factors.

. Classification

1. Decreased production due to drugs, radiation, myelopthisis, aplastic anemia, or platelet maturation defect (due to vitamin B_{12} or folate deficiency)
2. Abnormal sequestration of platelets in the spleen in congestive splenomegaly
3. Dilutional (e.g., massive blood transfusion)
4. Increased destruction, e.g., DIC, TTP, ITP, drugs, or malignancy

C. Idiopathic thrombocytopenic purpura (ITP)

1. **Clinical features.** ITP is characterized by an increased **peripheral platelet destruction** in the spleen, often **immune-mediated.** The course may be acute and self-limited (most common form in children), often following a viral infection, or it may be chronic (most common form in adults). ITP may be primary or secondary to another disorder such as SLE, HIV infection, or hemolytic anemia. The disease may present with a long history of easy bruisability, mucous membrane bleeding, gastrointestinal or genitourinary bleeding, and petechiae. CNS bleeding may occur.

D. Thrombotic thrombocytopenic purpura (TTP)

1. **Clinical features.** This is a rare disease characterized by thrombocytopenic purpura, fever, renal failure, neurological changes, and microangiopathic, hemolytic anemia. It appears most frequently in young women. The etiology is unknown.

PLATELET FUNCTION DEFECTS

A. **Clinical features.** These disorders are characterized by prolongation of the **bleeding time** in the presence of a normal platelet count.

B. **Classification.** Qualitative platelet defects may be congenital or acquired. They may be classified as follows:

1. **Defects of adhesion** (e.g., von Willebrand's disease, Bernard-Soulier disease)
2. **Defects of primary aggregation** (e.g., thrombasthenia)
3. **Defects of secondary aggregation and release** (e.g., aspirin, storage pool disease)

FLASHBACK TO PHYSIOLOGY

The steps in the formation of the platelet plug are discussed in detail in the Heme/Lymph Physiology section.

C. **von Willebrand's disease**

1. **Clinical features.** There is an autosomal dominant defect in von Willebrand's factor. This factor is necessary for adhesion of platelets to collagen. This results in **impaired platelet adhesion,** although the platelets themselves are intrinsically normal. It is characterized by spontaneous hemorrhage from mucous membranes, wounds, and excessive menstrual bleeding.
2. **Pathology.** Patients with von Willebrand's disease have a range of clinical syndromes but are usually diagnosed when they bleed after surgery or dental extraction. Von Willebrand's factor (vWF) is also the carrier molecule for factor VIII, so patients with vWF deficiency have low factor VIII levels and activity. As a result, they have a prolonged partial thromboplastion time (PTT) in addition to an elevated bleeding time.

DISORDERS OF EXCESS PLATELETS

These disorders are defined by the elevation of the platelet count above the normal range.

A. **Classification**

1. **Thrombocytosis** is a reactive disorder resulting from bleeding, hemolysis, inflammation, malignancy, iron deficiency, stress, or postsplenectomy.
2. **Essential thrombocythemia** is a primary **myeloproliferative disorder.** Thrombocythemia is also a prominent feature of chronic myelogenous leukemia (CML).

NOTE

Excess platelets do not necessarily cause thrombosis because in myeloproliferative disorders they may not function normally.

CLOTTING FACTORS DISORDERS

Clotting factors disorders are characterized by deficits of secondary hemostasis, due to alteration of the plasma protein factors of the clotting system.

A. **Clinical features.** Bleeding in disorders of secondary hemostasis tends to be from small arteries or into deep structures such as joint spaces or the retroperitoneum. Trauma may precede the bleeding but hemorrhage is often delayed.

B. **Laboratory values.** The most common blood tests to assay for the presence of an intact clotting system are the **prothrombin time (PT), partial thromboplastin time (PTT),** and **thrombin time (TT).**

1. PT measures factors (fibrinogen) I, II, V, VIII, and X.
2. PTT measures XII, prekallikrein, high-molecular weight, kininogen, and factors I, II, V, VIII, IX, X, and XI.
3. TT measures factor I (fibrinogen).

. **Hereditary deficiencies**

1. **Factor VIII:C deficiency (hemophilia A)** is an X-linked recessive disorder with an incidence of 1/10,000. It results from defective factor VIII:C or impaired conversion of precursor to factor VIII:C. Severe cases bleed in infancy at circumcision or may have multiple hemarthroses. Moderate cases have occasional hemarthroses. Mild cases may be missed until the patients bleed following a dental or surgical procedure. Bleeding may require treatment with cryoprecipitate or lyophilized factor VIII.
2. **Factor IX deficiency (Christmas disease, hemophilia B)** occurs approximately one-sixth as often as factor VIII deficiency. It is due to inactive or inadequate Factor IX and is also an X-linked recessive. The signs and symptoms are the same as hemophilia A. Since both hemophilia A and B have a prolonged PTT, these diseases must be distinguished by specific factor assays.

D. **Acquired disorders**

1. **Vitamin K deficiency.** Vitamin K is a fat-soluble vitamin produced by bacterial metabolism of ingested nutrients within the large intestine. It is essential in the **post-translational modification of factors II, VII, IX, and X,** as well as **proteins C and S.** Vitamin K deficiency may result from fat malabsorption, diarrhea, dietary deficiency (i.e., usually patients on parenteral feedings who are not receiving vitamin K supplements), antibiotics (which may kill gut flora), and some anticoagulant drugs (e.g., warfarin).
2. **Liver disease.** Factors II, V, VII, IX, X, XI, and XII are synthesized in the liver; liver disease can result in failure to synthesize these clotting factors, with a resultant bleeding diathesis.
3. **Disseminated intravascular coagulation (DIC)**
   a. **Clinical features.** This is an acquired **consumption deficiency of clotting factors and platelets,** often resulting in fatal thrombosis and hemorrhage. Coagulation system activation leads to microthrombus formation with consumption of platelets, fibrin, and clotting factors in the vasculature; this leads to activation of the fibrinolytic system. Hence, morbidity from DIC may be related to either thrombosis (tissue hypoxia and infarction) or hemorrhage (coagulation factor consumption and fibrinolysis).
   b. **Pathology.** There is diffuse thrombus formation, especially in the brain, heart, lung, kidneys, adrenals, spleen, and liver. There may be diffuse bleeding as well.

CLINICAL CORRELATE

Proteins C and S are involved in normal clot lysis. People with deficiencies of these proteins may develop frequent deep venous thrombosis. In addition, factor V resistant to protein C has recently been recognized as an inherited cause of deep venous thrombosis.

CLINICAL CORRELATE

A low factor VIII level may be used to distinguish DIC from the coagulopathy of liver failure, which has similar features except for a normal to elevated factor VIII level.

Note that factor VIII in synthesized in the endothelium of vessels; the other clotting factors are synthesized in the liver.

c. **Diagnosis.** DIC is diagnosed in the laboratory by demonstrating **low platelets, low fibrinogen,** and the presence of **fibrin degradation products.**

NON-NEOPLASTIC WHITE BLOOD CELL DISORDERS

A. **Leukopenia** is a decrease in the circulating WBC count. It may selectively involve one WBC line, such as lymphocytes (lymphopenia), or more commonly, neutrophils (neutropenia or granulocytopenia).

1. **Classification of neutropenias**
   a. **Decreased neutrophil production** is seen in megaloblastic anemias, aplastic anemia, some leukemias and lymphomas, drug suppression of myeloid stem cell differentiation, or autoimmune attack on stem cells.
   b. **Increased destruction of neutrophils** is usually due to splenic sequestration, which is often immune-mediated (e.g., Felty's syndrome).
   c. **Drug-induced neutropenia** may be seen in patients treated with alkylating agents, chloramphenicol, sulfonamides, chlorpromazine, and phenylbutazone. Mechanisms may include both decreased production and increased destruction. The problem is usually reversible if the drug is stopped.
2. **Clinical features** usually result from lack of immune defense provided by neutrophils.
   a. **Constitutional symptoms** include fever, chills, malaise, fatigability, and a high susceptibility to infection, particularly Gram-negative septicemia.
   b. **Prognosis** is often poor with death resulting from overwhelming infection; early diagnosis and antibiotic therapy for infections is required to avoid a fatal outcome.
3. **Pathology**
   a. **Bone marrow findings** depend on the etiology of the neutropenia. The neutropenia may be hypercellular due to increased destruction or megaloblastic anemia, or hypocellular, due to decreased production. RBC and platelet lines may be affected. There may be increased numbers of lymphocytes and plasma cells that result from relative preservation.
   b. **Infection.** Infected, necrotic ulcers may occur in the oral cavity, skin, vagina, anus, gastrointestinal tract, or, less commonly, in the lungs and urinary tract. Lymphadenopathy draining infected sites may be seen. Uninhibited by neutrophils, bacteria may form colonies.

. **Leukocytosis** is an increase in WBC count.

1. **Classification.** Leukocytosis may occur in a variety of WBC lines.
   a. **Monocytosis** may be seen in tuberculosis, endocarditis, malaria, brucellosis, rickettsiosis, and monocytic leukemia.
   b. **Lymphocytosis** may be seen in tuberculosis, brucellosis, viral hepatitis, cytomegalovirus infections, infectious mononucleosis, chronic lymphocytic leukemia (CLL), and some lymphomas.
   c. **Eosinophilic leukocytosis** may be seen in neoplasms, allergy, asthma, collagen vascular diseases, and parasitic infections. Any skin rash may produce eosinophilia.
   d. **Polymorphonuclear leukocytosis (most common)** may be seen in acute infection, tissue necrosis, and "stress," and may be accompanied by immature forms in the peripheral blood (leukemoid reaction or "left shift"). Chronic myelogenous leukemia (CML) produces extreme leukocytosis with immature forms and eosinophils as well as basophila.

C. **Nonspecific lymphadenitis**

1. **Clinical features.** Nonspecific lymphadenitis may be caused by drugs, toxins, or infection. It is common in the neck following dental or tonsillar infection, and in the axillae or the inguinal regions following infections of the extremities. Enlarged abdominal lymph nodes (mesenteric adenitis) may cause abdominal pain resembling acute appendicitis. Lymphadenopathy may be generalized in systemic viral or bacterial infections. A syndrome of generalized lymphadenopathy may be a precursor to AIDS. It is associated with hyperglobulinemia and normal CD4 lymphocyte counts.

LYMPHOMAS

A. **Hodgkin's disease (Hodgkin's lymphoma)**

1. **Overview.** Hodgkin's disease is classically considered separately from other lymphomas (nonHodgkin's lymphomas) because its **spread is almost always in contiguity** (i.e., from one set of lymph nodes to the next). The spleen is involved before the liver. It almost never has a leukemic component. It has a high cure rate, and, histologically, it is characterized by the presence of the **Reed-Sternberg giant cell** (RS cell). The RS cell is large (15-45 m), often with two or more nuclei, containing large "owl-eyed" nucleoli surrounded by a clear halo; cytoplasm is abundant (Figure 10-1). The presence of the RS cell is

NOTE

Leukocyte alkaline phosphatase is elevated in inflammatory leukocytosis. It is depressed in chronic myelogenous leukemia.

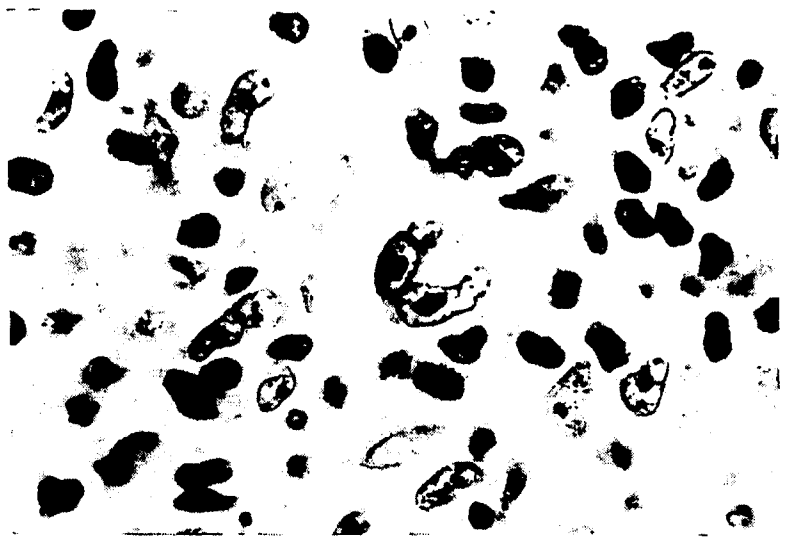


Figure 10-1. Reed-Sternberg cell (microscopic).

necessary but not sufficient to make the diagnosis of Hodgkin's disease.

2. **Clinical features.** Hodgkin's disease may present with painless cervical adenopathy or with constitutional (hypermetabolic) symptoms: fevers, chills, night sweats, and weight loss.
3. **Pathology.** There are four variants recognized. In order of best prognosis to worst:
   a. **Lymphocyte predominance** shows a sea of lymphocytes with few RS cells, a variable number of histiocytes, little fibrosis, and no necrosis.
   b. **Nodular sclerosis** is more common in women and tends to involve mediastinal, supraclavicular, and lower cervical nodes. There is a mixed infiltrate composed of lymphocytes, histiocytes, a few eosinophils, plasma cells, and RS cells.
   c. **Mixed cellularity** shows a mixture of neutrophils, lymphocytes, eosinophils, plasma cells, and histiocytes. Many classic RS cells may be identified.
   d. **Lymphocyte depletion** shows rare lymphocytes and many RS cells with variable eosinophils, plasma cells, and histiocytes. Diffuse fibrosis may be seen.
4. **Course and prognosis** depends on multiple factors, including age (younger do better), the presence or absence of constitutional symptoms, histology (patients with lymphocyte predominance and nodular sclerosis do better than patients with mixed cellularity or lymphocyte depletion), and stage (the lower the stage, the better).

NonHodgkin's lymphomas (NHL)

1. **Overview.** This is a varied group of lymphoreticular neoplasms usually characterized by **lymphadenopathy** and **hepatosplenomegaly.** In most cases the disease is first discovered in only one chain of nodes—usually cervical, axillary, inguinal, femoral, iliac, or mediastinal. Unlike Hodgkin's disease, NHL do not produce RS cells, they generally do not spread in contiguity, and they frequently have a leukemic or blood-borne phase.
2. **Incidence.** The peak incidence is in the late 50s. NHLs are rare and more aggressive in children and young adults. They may involve lymph nodes or lymphoid tissue in the gut, oropharynx, liver, spleen, and thymus. Presentations include local or generalized lymphadenopathy, abdominal or pharyngeal mass, abdominal pain, or gastrointestinal bleeding. Weight loss is common and is a sign of disseminated disease. NHLs are common in immunosuppressed patients, whether iatrogenic, congenital, or aquired as in AIDS.
3. **Types of NonHodgkin's lymphomas**
   a. **Well-differentiated lymphocytic lymphoma (WDLL)**
      (1) **Clinical features.** WDLL comprises approximately 5% of NHLs and is usually diffuse. It usually affects older patients who present with generalized lymphadenopathy and mild hepatosplenomegaly.
   b. **Poorly differentiated lymphocytic lymphoma (PDLL)**
      (1) **Clinical features.** PDLL comprises approximately 30% of NHLs. It may be nodular or diffuse. Patients are usually middle-aged or older. In 75% of cases, they present with lymphadenopathy and infiltration of bone marrow, liver, and spleen at the time of diagnosis.
   c. **Histiocytic lymphoma**
      (1) **Clinical features.** This is one of the most common NHLs. It is usually diffuse but may be nodular. Diffuse histiocytic lymphoma (DHL) may present with nodal involvement (usually on one side of diaphragm), extranodal involvement (gastrointestinal tract, skin, brain, bone), or, rarely, liver and spleen involvement.
   e. **Lymphoblastic lymphoma**
      (1) **Clinical features.** MLHL is often associated with a mediastinal mass (thymoma), suggesting a thymic origin for the neoplastic cells. These cells often express T-cell markers.
   f. **Undifferentiated lymphoma: Burkitt's**
      (1) **Clinical features.** This disease is endemic in Africa and sporadic in the United States. It usually affects children or young adults. Lymphadenopathy is a rare initial pre-

NOTE

The term "histiocytic" is actually a misnomer: the tumors are composed of monoclonal B cells, not cells of macrophage lineage.

sentation. In Africa it often arises in the mandible or maxilla; in the United States, it often arises in the abdomen (gastrointestinal tract, ovaries, retroperitoneum). The etiology of Burkitt's lymphoma is thought to be related to **Epstein Barr virus (EBV).** EBV may act as a mitogen, initiating a sustained polyclonal activation of B cells. This eventually results in a neoplastic proliferation of a single B-cell clone after a chromosomal translocation occurs.

(2) **Pathology.** There is a uniform sea of moderately large cells with round nuclei, multiple nucleoli, moderate basophilic cytoplasm with lipid-containing vacuoles, frequent mitoses, and many macrophages with ingested debris, producing the so-called **"starry sky" pattern.** A leukemic phase is rare.

LEUKEMIAS

The leukemias are a group of malignant neoplasms of WBC precursors characterized by abnormal leukocytes in the peripheral blood, liver, spleen, and bone marrow. Most of the morbidity results from the functional impairment of WBC, RBC, and platelets, leading to infection, anemia, and bleeding.

A. Classification

1. **Acute leukemias** are characterized by the presence of **blasts in the peripheral blood** and lack of mature cells. They are usually rapidly fatal if left untreated (2-4 months). The two most common types are **acute lymphocytic leukemia (ALL)** and **acute myelogenous leukemia (AML). Acute monocytic leukemia (AMoL)** and **acute undifferentiated leukemia (AUL)** occur less frequently.
2. **Chronic leukemias** are characterized by elevated numbers of more mature leukocytes in the peripheral blood. They have a longer course (if untreated, 3-10+ years). **Chronic myelogenous leukemia** (CML) and **chronic lymphocytic leukemia (CLL)** are the two most common forms. **Chronic monocytic leukemia (CMoL)** is much less common.

B. Acute lymphocytic leukemia (ALL)

1. **Clinical features.** ALL accounts for 60%-70% of childhood leukemia. The peak incidence is at age 4; it is rare over age 50. ALL presents with fatigue, fever (secondary to neutropenia and infection), bleeding in the form of epistaxis, gingival petechiae, ecchymoses (secondary to thrombocytopenia); subarachnoid or cerebral hemorrhage may occur. Patients may

IN A NUTSHELL

Acute leukemias have blasts in peripheral blood and decreased mature cells, while chronic leukemias have an increased number of mature WBCs in the peripheral blood.

have lymphadenopathy, hepatosplenomegaly, or bone pain from infiltration of these areas.

2. **Pathology**
   a. Almost all patients present with anemia and thrombocytopenia on peripheral smear. The initial WBC count is variable and may be high, normal, or low, depending on the course of the disease. **Lymphoblasts** are prominent and mature WBCs are rare.

D. **Acute myelogenous leukemia (AML)**

1. **Clinical features.** AML represents approximately 20% of acute leukemia in children and is the most common acute leukemia in adults. Signs and symptoms resemble ALL, except that AML usually presents with lymphadenopathy or splenomegaly.
2. **Pathology.** One may see tissue infiltrates of neoplastic cells called chloromas. The primary cell type is variable.
   a. **Myeloblasts** have a round-oval nucleus, loose chromatin, two or more nucleoli, and pale blue cytoplasm. They may contain **Auer rods** (finely granular cytoplasmic bodies), which are abnormal fused lysosomal structures.

E. **Chronic myelogenous leukemia (CML)**

1. **Clinical features.** This is primarily a disease of middle age but may occur in children and young adults. Initial symptoms are often fatigue, fever, night sweats, weight loss. **Splenomegaly** is common and often massive enough to cause abdominal discomfort. Laboratory studies may show marked leukocytosis (50,000-500,000), **low-to-absent leukocyte alkaline phosphatase,** elevated serum vitamin B_{12} and vitamin B_{12}-binding proteins, and high uric acid as a result of rapid cell turnover. After a variable remission period, patients may develop **blast crisis,** which is an acute resistant form of leukemia, leading to death. Approximately two-thirds of patients convert to AML and one-third to B-cell ALL.
2. **Pathology**
   a. The peripheral smear shows a very high WBC count. Segmented neutrophils, metamyelocytes, and myelocytes are predominant, but promyelocytes and blasts are also present. Eosinophils and basophils are present and often prominent. Lymphocytes are few. There is a moderate anemia with some anisocytosis. Platelets are usually increased, often markedly.
   b. The bone marrow is packed, often 100% cellular with hyperplasia of the granulocytic cell line.

c. The **spleen may be massively enlarged** (up to 5 kg). Leukemic cells may obstruct vessels, leading to multiple splenic infarcts.

d. Over 95% of patients with CML have the **Philadelphia chromosome (Ph^1)**, the result of translocation from the long arm of chromosome 22 to chromosome 9 in all dividing progeny of pluripotent stem cells.

F. **Chronic lymphocytic leukemia (CLL)**

1. **Clinical features.** This is a disease of patients usually over 60 years of age. It is common in the West and rare in Asians. There is usually an insidious onset, often discovered incidentally during routine blood testing. The patient may be asymptomatic or present with fatigue and weight loss; lymphadenopathy and hepatosplenomegaly are later findings. Patients may develop low levels of gamma globulins with resultant susceptibility to infection. Patients with CLL are also thought to have a higher incidence of visceral malignancy (e.g., gastrointestinal tract, lung, skin), and develop autoimmune hemolytic anemia more frequently than the normal population.

2. **Pathology**

a. The peripheral smear shows marked lymphocytosis (50,000-250,000). Normochromic, normocytic anemia is common and autoimmune hemolysis may occur. Platelets are initially normal, then decrease as the bone marrow is replaced by neoplastic cells.

G. **Adult T-cell leukemia/lymphoma**

1. **Clinical features.** This disease is endemic in Japan and sporadic in the West. It is caused by the **human T-cell leukemia/lymphoma virus (HTLV 1),** a virus with some similarities to human immunodeficiency virus (HIV). Patients present with lymphadenopathy, hepatosplenomegaly, skin involvement, and hypercalcemia. The incubation period after exposure to the virus may be decades.

2. **Pathology.** The primary cell type is the CD4 T cell.

PLASMA CELL DYSCRASIAS

A. **Hypergammaglobulinemia** is an increased serum level of immunoglobulin.

1. **Classification**

a. **Monoclonal immunoglobulin molecules,** or M components, belong to a single class, subclass, and type. Although complete immunoglobulin molecules circulate in the plasm

NOTE

The classical CLL cell is the CD5 B cell. CLL cells divide very slowly but do not undergo apoptosis and so accumulate endlessly.

IN A NUTSHELL

Leukemia clues:

- *Children/lymphoblasts* → *ALL*
- *Myeloblasts* → *AML*
- *Auer rods* → *AML, promyelocytic*
- *DIC* → *Promyelocytic*
- *Elderly* → *CLL*
- *Massive splenomegaly* → *CML*
- *Philadelphia chromosome* → *CML*
- *HTLV-1* → *Adult T-cell*

and interstitium, fragments, such as immunoglobulin light chains, may be found in the urine. **Monoclonal gammopathies** may be malignant (e.g., multiple myeloma, Waldenström's macroglobulinemia, heavy-chain disease) or benign (e.g., monoclonal gammopathy of undetermined significance, MGUS).

b. **Polyclonal immunoglobulins** are usually due to antigenic stimulation. Liver disease also elevates immunoglobulins as a result of decreased catabolism. Polyclonal hypergammaglobulinemia typically occurs 1-2 weeks after an antigen stimulus. It may follow bacterial infection, or occur with granulomatous disease, connective tissue disorders, and liver failure secondary to decreased catabolism. Pathology is variable and depends on the underlying disorder.

2. **Laboratory tests** show elevated serum globulins, an elevated erythrocyte sedimentation rate (ESR), and a positive serum protein electrophoresis. **Bence-Jones proteins** (free light chains) may be found in serum or urine. In myeloma, the free light chains are monoclonal. In inflammation, liver disease, or glomerulopathy, the free light chains are polyclonal.

3. **Clinical features**

a. **Hyperviscosity of blood** may lead to sludging and rouleaux formation with subsequent thrombosis, hemorrhage, renal impairment, CNS disturbances, and right-sided congestive heart failure.

b. **Cryoglobulins,** immunoglobulins that precipitate in the cold (usually M components), may lead to Raynaud's phenomenon, thrombosis, and gangrene.

c. **M components** may interfere with clotting, leading to gastrointestinal or retinal hemorrhage. They most often inhibit factors IX and X.

d. **The presence of immunoglobulins** with antibody activity in the serum may lead to immune-mediated destruction of RBCs, granulocytes, or platelets with resultant anemia, granulocytopenia, or thrombocytopenia.

B. **Multiple myeloma**

1. **Clinical features.** Multifocal plasma cell neoplasms in the bone marrow and, occasionally, soft tissues, produce **monoclonal immunoglobulins (IgG).** Signs and symptoms result from excess abnormal immunoglobulins causing hyperviscosity, and from infiltration of various organs by neoplastic plasma cells. Immune-mediated destruction of blood cells and lack of normally functioning antibodies lead to **susceptibility to infection. Proteinuria** may contribute to progressive renal failure.

Infiltration of bone with plasma cell neoplasms may lead to **bone pain** and **hypercalcemia.** Over 99% of patients have elevated levels of serum immunoglobulins or urine Bence-Jones proteins, or both. **Serum protein (SPEP) electrophoresis** shows a homogeneous peak or "spike."

2. **Pathology**
   a. **Bone.** The marrow is infiltrated with **plasma cells** (usually over 30%) in various stages of maturation, called "myeloma cells." They may resemble lymphoid precursors with cytoplasmic inclusions (acidophilic aggregates of immunoglobulin) called Russell bodies. Multiple **osteolytic lesions** throughout the skeleton, resulting from plasma cell secretion of osteoclast activating factor (OAF), appear as **"punched-out" defects on x-ray.** These lesions most commonly involve vertebrae, ribs, skull, pelvis, femur, clavicle, and scapula (Figure 10-2, 10-3).
   b. **Kidney.** Interstitial plasma cell or chronic inflammatory infiltrate may be seen. Protein casts in distal tubules result from glomerular damage by M proteins.
   c. **Nervous system.** Tumor infiltrating nerve roots or vertebral compression fractures may lead to neuropathy or myelopathy.
   d. **Other.** Ten percent of patients develop amyloidosis, more often with λ than κ chains. Plasma cell infiltrates may be found in lymph nodes, liver, and spleen.

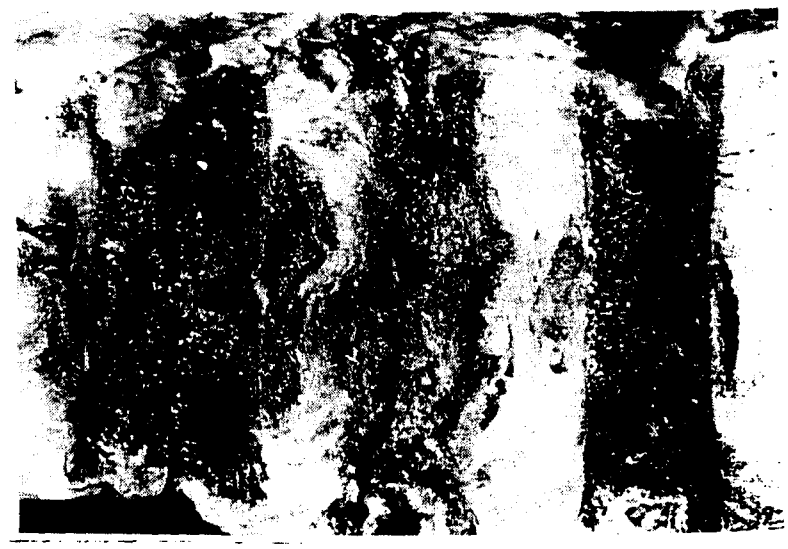


Figure 10-2. Vertebral column in multiple myeloma (gross).

IN A NUTSHELL

Classic clues for multiple myeloma:

- *Monoglonal gammopathy–single spike on SPEP*
- *Proteinuria with Bence-Jones protein in urine*
- *Bone pain and hypercalcemia*
- *"Punched out" or "moth-eaten" lytic bone lesions on x-ray*

Figure 10-3. Multiple myeloma with bone resorption (microscopic).

C. **Waldenström's macroglobulinemia**

1. **Clinical features.** These are neoplasms of lymphocytoid plasma cells that produce **monoclonal IgM.** The disease resembles lymphocytic lymphoma. Symptoms are due to hypergammaglobulinemia and tumorous infiltration. Most patients present with constitutional symptoms (i.e., fatigue, weakness, weight loss) but they may present with hepatosplenomegaly, lymphadenopathy, bone pain, and manifestations of hyperviscosity. Ten percent of patients have Bence-Jones proteins in urine. Almost all patients have an M-protein spike on serum protein electrophoresis.
2. **Pathology**
   a. **Bone marrow** shows infiltrates of lymphocytes, plasma cells, lymphocytoid plasma cells, and related variants. There is **no bone erosion.**

D. **Monoclonal gammopathy of undetermined significance (MGUS)**

1. **Clinical features.** There is an asymptomatic elevation in serum immunoglobulins detected as an M-protein spike on serum electrophoresis.
2. **Pathology.** Patients have a low concentration of M protein, and the bone marrow contains less than 5% plasma cells.
3. **Prognosis.** This disorder was initially thought to be benign, but approximately 2% of patients a year with MGUS may later develop myeloma, lymphoma, amyloidosis, or Waldenström's macroglobulinemia.

NOTE

A major difference between multiple myeloma and Waldenström's macroglobulinemia is the lack of lytic bone lesions in Waldenström's.

DISORDERS OF THE SPLEEN

A. **Splenomegaly**

1. **Clinical features.** There are multiple etiologies for splenomegaly, including infections (e.g., mononucleosis, TB, CMV, malaria), congestion with portal hypertension (e.g., cirrhosis, portal vein thrombosis, right-sided CHF), inflammation (e.g., SLE and rheumatoid arthritis), lymphohematogenous disease (e.g., myeloma, lymphoma, leukemia), storage disease (e.g., Gaucher's, Neimann-Pick's), and others (e.g., infarcts, amyloid, tumor).
2. **Signs and symptoms**. The patient may complain of left upper quadrant discomfort. Sequestration of blood elements by an enlarged spleen is known as **hypersplenism** and is characterized by splenomegaly, reduction of one or more blood cell lines with resultant anemia, leukopenia, or thrombocytopenia, and resolution of the blood disturbance by splenectomy.
3. **Pathology** depends on the underlying disease. Congestive splenomegaly may lead to a large, firm, red spleen with a thick capsule.

B. **Splenic infarcts**

1. **Clinical features.** Infarcts initially present with splenomegaly, but then fibrosis and shrinkage occur. They may be caused by occlusion of the splenic artery or its branches, but are most commonly caused by **embolisms from the heart;** thrombosis may occur. Occlusion of sinusoids by **sickled cells** also produces multiple microinfarcts, leading to autosplenectomy.
2. **Pathology**. Infarcts may be single or multiple, small or large. They are usually **pale and wedge-shaped** with a broad base at the periphery. Suppurative necrosis may develop, followed by scarring.

C. **Splenic neoplasms**

1. **Clinical features.** Most tumors involving the spleen are lymphohematogenous neoplasms, but others may occur.
2. **Primary neoplasms**
   a. **Benign.** Fibromas, osteomas, chondromas, lymphangiomas, and hemangiomas (often cavernous) are all rare.
   b. **Malignant.** Lymphomas are by far the most common; hemangiosarcomas are rare.

CLINICAL CORRELATE

Recall that patients undergoing splenectomy should receive vaccinations to protect them from encapsulated organisms. Vaccines for pneumococcus and H. influenzae are available.

Rupture

1. **Clinical features.** Rupture is usually due to trauma but may be spontaneous in leukemia, malaria, typhoid fever, and infectious mononucleosis.
2. **Pathology.** There is usually massive intraperitoneal hemorrhage. The peritoneal cavity may be seeded with foci of splenic tissue.

Congenital anomalies of the spleen, including accessory spleens and abnormal lobulations, are common. Small accessory spleens can become clinically significant when they enlarge following a therapeutic splenectomy.

DISORDERS OF THE THYMUS

A. **Atrophy of the thymus** may be seen in several congenital abnormalities and with radiation, chemotherapy, and stress.

1. **Pathology**
   a. **Congenital immune deficiencies** as a result of adenosine deaminase deficiency or interleukin-2 (IL-2) receptor deficiency lead to the absence of lymphoid precursors and failure of the thymus to populate with thymocytes. **Severe combined immunodeficiency disorder** (SCID) results.
   b. **Other congenital immune deficiencies,** such as **Nezeloff's syndrome** and **DiGeorge syndrome,** lead to atrophy or failure to develop a thymus. In DiGeorge syndrome, a defect in the development of the third and fourth pharyngeal pouches also leads to the absence of the parathyroids, causing parathormone deficiency and tetany shortly after birth.

B. **Hyperplasia of the thymus** with germinal center formation may be seen in some autoimmune diseases, such as **myasthenia gravis,** where antibodies are made to acetylcholine receptors. Removal of the thymus often cures the disease.

C. **Epithelial thymomas** are benign in 90% of cases.

1. **Pathology.** Most epithelial thymomas are lobulated and encapsulated and are composed of a mixture of epithelial cells and T lymphocytes.
2. **Clinical features.** The mean age is 50. They may present incidentally on chest x-ray with cough, dyspnea, or dysphagia. There is an increased incidence of thymoma in patients with myasthenia gravis.

FLASHBACK TO EMBRYOLOGY

Remember that the 3rd pharyngeal pouch gives rise to the thymus gland while the 4th pouch gives rise to the superior parathyroids.

D. **Lymphomas** may originate in the thymus. The thymus may also be secondarily involved in Hodgkin's disease, nonHodgkin's lymphomas, and acute lymphoblastic leukemia.

Musculoskeletal, Connective Tissue, and Integument Pathology

Musculoskeletal and skin pathology encompass a broad range of congenital anomalies, autoimmune diseases, and neoplastic disorders. This chapter will discuss the characteristics of each disease state, their clinical presentations, and, when identifiable, their causes and treatments.

BONE

A. **Congenital anomalies**

1. **Osteogenesis imperfecta** is a term used to describe several clinical phenotypes of hereditary bone fragility.
   a. **Four types** are generally recognized. All are rare.
      (1) Type I shows autosomal dominant inheritance and causes mild-to-moderate long bone disease, blue sclerae, deafness, and little progression after puberty.
      (2) Type II is autosomal recessive and often produces a stillborn infant, or death after birth, with generalized crumpled bones.
      (3) Type III is autosomal recessive and produces progressive severe deformity. Patients have white sclerae.
      (4) Type IV is autosomal dominant with variable severity, normal sclerae, and fractures of the long bones and spine.
   b. **Etiology.** The cause in all cases of osteogenesis imperfecta seems to be a **defect in the synthesis of type I collagen.**
   c. **Pathology**
      (1) In bones, woven bone instead of trabecular bone and abnormal arrangements of collagen fibers are seen. Joints show **ligamentous laxity** as a result of abnormal collagen.
      (2) In the eye, some patients have an abnormally thin **sclera with a blue hue.**

CLINICAL CORRELATE

Osteogenesis imperfecta is a disease of type I collagen synthesis. Heritability varies among the types; blue sclerae and lax ligaments are common features.

(3) In the ears, there may be fractured ossicles, producing deafness.

(4) Teeth may be small and discolored (dentinogenesis imperfecta), there may be mitral valve prolapse, and the dermis may be abnormally thin.

2. **Osteopetrosis** is a group of hereditary disorders characterized by **increased density and thickening of bone cortex** with narrowing of medullary cavities. Bones are brittle and fracture easily. Membranous bones are not affected (e.g., cranium). It may be associated with anemia, blindness, deafness, hydrocephalus, and cranial nerve palsies. There are two forms of inheritance.
   a. **Autosomal recessive disease** affects children and produces early death due to anemia as the bones grow and squeeze out the marrow space.
   b. **Autosomal dominant disease** affects adults and does not cause death but may cause increased fractures and encroach upon cranial nerves as they exit from the skull.

NOTE

The defect in osteopetrosis seems to be an inability of osteoclasts to resorb bone.

3. **Achondroplasia** is an autosomal dominant disease characterized by **abnormal cartilage synthesis** with subsequent decreased epiphyseal bone formation. It spares the cranium and vertebral bones. Clinically, achondroplasia is characterized by **dwarfism** with short extremities and a large body and head.

CLINICAL CORRELATE

Achondroplasia is the best known form of dwarfism, characterized by short limbs, large body, frontal bossing, and "saddle nose."

4. **Osteochondromatosis** is a hereditary disorder characterized by the formation of multiple **exostoses.**
   a. **Clinical features**
      (1) Exostoses may be asymptomatic or produce deformity and compromise the blood supply.
      (2) **Gardner's syndrome** is a rare genetic disorder in which there is an association of exostoses with sebaceous cysts, desmoid tumors, and colonic polyps, which may become carcinomas.
   b. **Pathology.** Exostoses are bony metaphyseal projections capped with cartilage. They are multiple, often symmetric, and originate from epiphyseal cartilage.
5. **Enchondromatosis** (Ollier's disease) is a nonhereditary syndrome characterized by multiple **cartilaginous masses within the medullary cavity of bone,** most commonly in the hands and feet. It often presents with pain and fractures. These masses may undergo malignant transformation; half of all chondrosarcomas arise from enchondromas. **Mafucci's syndrome** is a familial association of enchondromas and hemangiomas of the skin.

. **Osteoporosis** is a decrease in bone mass, causing fragility of bone. Osteoporosis most commonly occurs in postmenopausal women.

1. **Pathogenesis**
   a. **Primary causes** include estrogen deficiency, low density of original bone, lack of exercise, and nutritional factors associated with accelerated bone loss.
   b. **Secondary causes** include immobilization, endo-crinopathies (e.g., Cushing's, thyrotoxicosis), and malnutrition (e.g., deficiencies of calcium, vitamins C and D, protein).
2. **Clinical features**
   a. Patients may experience **pain and fractures** without obvious trauma.
   b. X-rays show generalized **radiolucency** of bone.
   c. Laboratory tests reveal normal serum calcium, phosphorus, and alkaline phosphatase.
3. **Pathology.** Thinned cortical bone and an enlarged medullary cavity are seen. All bones are affected. Weight-bearing bones (vertebrae, femoral neck) are predisposed to fractures. There is normal bone histology and a normal ratio of mineral/organic bony elements (Figure 11-1).

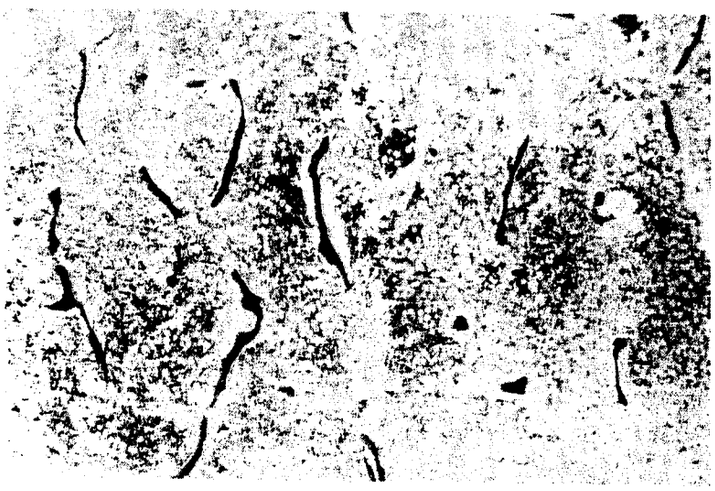


Figure 11-1. Osteoporosis of vertebra (microscopic).

NOTE

In osteoporosis, the bone is formed normally, but in decreased amounts.

IN A NUTSHELL

Osteoporosis may lead to easy fracturing, especially of hips and vertebrae.

C. **Osteomalacia and rickets**

1. **Etiology.** Both diseases are caused by **vitamin D deficiency** from chronic renal insufficiency, intestinal malabsorption, or dietary deficiency.
2. **Clinical features**
   a. **Rickets** occurs in children prior to closure of the epiphyses, leading to bone deformities and pain. Patients show the "rachitic rosary" (deformity of the chest wall as a result of swelling at osteochondral junctions of ribs), bowing of legs, and fractures.
   b. **Osteomalacia** is an impaired mineralization of the osteoid matrix. It causes fractures and bending of bones and widening of osteoid seams.
      (1) Laboratory tests show **low serum calcium and phosphorous** and **high alkaline phosphatase,** which distinguishes this syndrome from osteoporosis.
      (2) X-rays show diffuse radiolucency of bone.

D. **Paget's disease** (osteitis deformans) is due to **excessive bone** resorption with replacement by soft, poorly mineralized matrix in a disorganized array.

1. **Clinical features**
   a. Paget's disease may present with pain, deformity, and fractures. It is usually polyostotic (affecting many bones), involving the skull, pelvis, femur, and vertebrae. When the skull is involved, impingment of cranial nerves often causes deafness. Involvement may cause bone hypervascularity with increased warmth of the overlying skin.
   b. X-ray shows enlarged, radiolucent bones.
   c. Laboratory tests show an **extremely elevated alkaline phosphatase.**
2. **Pathology.** The disease progresses from an osteolytic to an osteoblastic process. Resorbed bone is replaced by a vascular connective tissue, which later becomes mineralized. There is a mosaic rather than trabecular pattern from persistent osteoid seams at the margin of new bone.

E. **Fibrous dysplasia** causes focal areas of fibrous replacement of bone. Incidence is higher in teenagers, with men more frequently affected than women.

1. **Clinical features.** Monostotic fibrous dysplasia is often asymptomatic or may lead to pathologic fracture. **Albright's syndrome** is an association of polyostotic fibrous dysplasia, café au lait spots, and sexual precosity in women.

NOTE

Rickets and osteomalacia are disorders of osteoid mineralization; osteoid is produced in normal amounts but is not calcified properly.

2. **Pathology.** Fibrous dysplasia is usually monostotic, affecting the long bones, ribs, skull, and facial bones. Fibrosis starts within the medullary cavity and remains encased in cortical bone.

Bone abnormalities in hyperparathyroidism (osteitis fibrosa cystica)

1. **Pathogenesis.** Excess parathyroid hormone activates osteoclasts to resorb bone and causes the kidney to waste calcium.
2. **Clinical features.** Osteitis fibrosa cystica occurs more commonly in primary hyperparathyroidism, causing bone pain and fractures.
3. **Pathology**
   a. **Microscopically**, there is an increased number of osteoclasts with excess bone resorption and fibrous replacement of marrow, causing cystic spaces in trabecular bone and **"brown tumors"** (areas of organized hemorrhage).
   b. **Grossly,** brown tumors may produce cystic enlargements of bones.

NOTE

Brown tumors are classical signs of hyperparathyroidism.

. **Hypertrophic osteoarthropathy**

1. **Clinical features**
   a. Clinically, hypertrophic osteoarthropathy presents with painful swelling of wrists, fingers, ankles, knees, or elbows. The pathogenesis is unknown.
   b. This is a **periosteal inflammation,** and new bone forms at the ends of long bones, metacarpals, and metatarsals.
   c. Arthritis of adjacent joints is commonly seen, often with digital clubbing.
2. **Etiology.** Causes include intrathoracic carcinoma (a paraneoplastic syndrome), sepsis, endocarditis, cyanotic congenital heart disease, and inflammatory bowel disease. The syndrome regresses when the underlying disease is treated.

1. **Fibrous cortical defect** (nonossifying fibroma) is a common developmental abnormality seen in bones of the lower extremities in children. They are non-neoplastic lesions of bone cortex that are composed of fibrous connective tissue.

1. **Clinical features.** Fibrous cortical defect is usually asymptomatic, non-neoplastic, and usually resolves spontaneously.
2. **Pathology.** There are irregular, well-demarcated, radiolucent defects in the bony cortex, with an intact subperiosteal shell of bone. In the metaphysis, there are whorls of connective tissue. Occasionally, multinucleated giant cells are seen. This entity must be differentiated from giant cell tumors of bone, which may cross the epiphysis (fibrous cortical defect does not do this). The stromal cells of giant cell tumors are also more

atypical, with larger, darker nuclei and less cytoplasm than seen in fibrous cortical defect.

I. **Osteomyelitis**

1. **Pyogenic osteomylitis**

a. **Etiology.** Caused by direct innoculation of bone or by seeding of bone after bacteremia. Organisms include ***Staphylococcus aureus,*** Streptococcus, Gonococci, and *Haemophilus influenzae.* Salmonella may be seen in patients with sickle cell disease. Pseudomonas is common in intravenous drug users and diabetics.

b. **Clinical features** include fever, localized pain, erythema, and swelling. The x-ray may be normal for up to 2 weeks, then may initially show periosteal elevation.

c. **Pathology.** Suppuration begins within the metaphyseal medullary cavity and penetrates the cortex. Compression by exudate leads to vascular insufficiency and ischemic necrosis. Specific findings include:

(1) **Sequestrum,** a necrotic bone fragment

(2) **Involucrum,** new bone that surrounds the area of inflammation

(3) **Brodie's abscess,** localized abscess formation in the bone

2. **Tuberculous osteomyelitis** occurs in 1% of cases of TB, causing caseating granulomas in the bones. The term **"Pott's disease"** refers to spinal involvement.

J. **Tumors**

1. **Osteoblastic tumors**

a. **Osteoma** is a **benign** growth that frequently involves the skull.

(1) "Hyperostosis frontalis interna" describes an osteoma that extends into the orbit or sinuses.

(2) Pathology shows dense normal bone.

b. **Osteoid osteoma** is a benign growth of the diaphysis of long bones, often the tibia or femur.

(1) **Clinical features** include pain that is worse at night and relieved by aspirin. X-rays show a central radiolucency surrounded by a sclerotic rim.

(2) **Pathology** shows a 1-cm brown nodule surrounded by dense sclerotic cortical bone. **Microscopically,** the nodule is formed of vascular, woven bone with partially mineralized osteoid.

c. **Osteoblastoma** is similar to a large osteoid osteoma, but is large, painless, often involves vertebrae, and may be malignant.

d. **Osteosarcoma** is a **malignant** bone tumor that produces osteoid and bone.

(1) **Incidence.** Men are affected more often than women, and the tumor usually occurs in the second and third decade of life. It is the most common bone tumor in older people and is often associated with Paget's disease. Over one third of patients with retinoblastoma also develop osteosarcoma.

(2) **Pathogenesis** is unclear. There is an increased incidence with irradiation, Paget's disease, and other previous bone pathology.

(3) **Clinical features.** Patients present with localized pain and swelling, weight loss, and anemia. Classic x-ray findings include **Codman's triangle** (periosteal elevation) and bone destruction.

(4) **Pathology. Grossly,** osteosarcoma, particularly in teenagers, often affects the metaphyseal ends of long bones, usually around the knee, producing large necrotic and hemorrhagic mass. **Microscopically,** the tumor may be sclerotic (with mineralized osteoid) or osteolytic (with little osteoid). It also may contain collagen or cartilage. The classic finding is **anaplastic cells with osteoid,** pink, amorphous material that is variably mineralized (Figures 11-2 and 11-3).

(5) **Prognosis is poor.** Patients are treated with amputation and chemotherapy. Metastasis to the lungs is common. Prognosis is improved with aggressive management, such as resecting single pulmonary metastases.

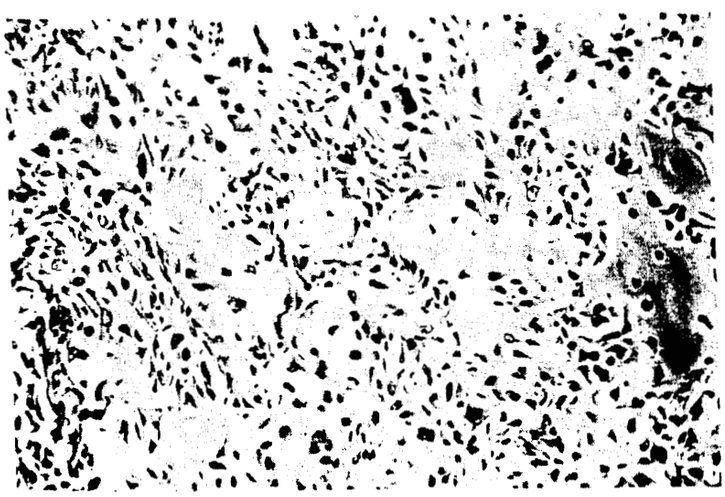


Figure 11-2. Osteogenic sarcoma (microscopic).

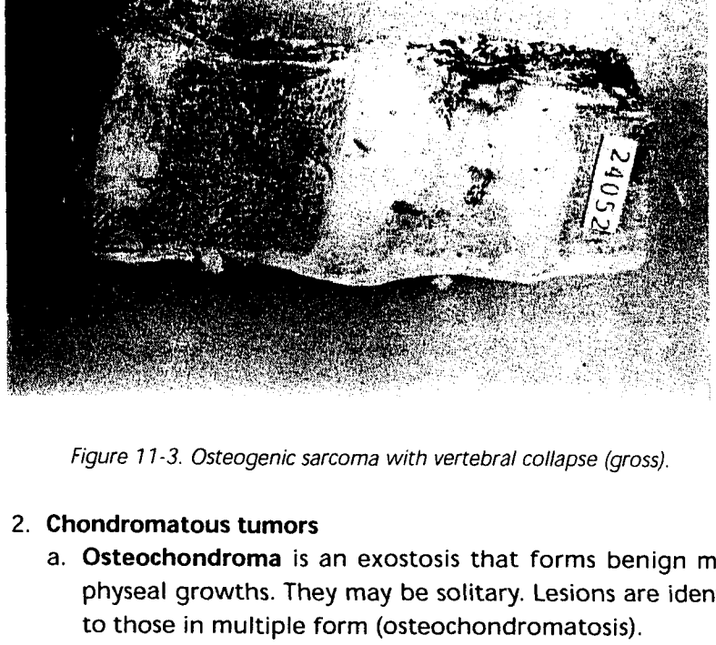


Figure 11-3. Osteogenic sarcoma with vertebral collapse (gross).

2. **Chondromatous tumors**
   a. **Osteochondroma** is an exostosis that forms benign metaphyseal growths. They may be solitary. Lesions are identical to those in multiple form (osteochondromatosis).
   b. **Enchondroma** is a solitary cartilaginous growth within the spongiosa of bone. Solitary growths are similar to those in multiple form (Ollier's disease).
   c. **Chondromyxoid fibroma** is a benign, rare tumor affecting young men. It forms a firm mass within the metaphyseal marrow cavity of the tibia or femur. The tumor contains fibrous and myxomatous tissue, which must be differentiated from a malignant lesion.
   d. **Chondrosarcoma** is a malignant tumor of chondroblasts. The age range is from 30-60 years. Men are more often affected than women.
      (1) **Etiology.** The tumor may arise *de novo* or secondary to a pre-existing enchondroma or exostosis.
      (2) **Clinical features.** Chondrosarcomas are slower growing than osteosarcomas. They typically present with pain and swelling.
      (3) **Pathology.** Tumors typically involve the spine, pelvic bones, and upper extremities. **Microscopically,** they are characterized by atypical chondrocytes and chondroblasts, often with multiple nuclei in a lacuna.
3. **Giant cell tumor** is a malignant neoplasm containing multinucleated giant cells and atypical stromal cells. This is an uncommon tumor, affecting patients from age 20-50 years.

IN A NUTSHELL

- ***Osteochondroma:*** *Exostosis from misdirected growth of growth plate*
- ***Enchondroma:*** *Solitary benign growth of cartilage inside bone*
- ***Chondrosarcoma:*** *Malignant cartilage-producing tumor*

a. **Clinical features**
   (1) Tumors present as a bulky mass with pain and tenderness.
   (2) X-rays show an expanding area of radiolucency without a sclerotic rim.

b. **Pathology**
   (1) **Grossly,** tumors arise in the epiphyseal region of long bones, forming a club-like deformity at the end of the bone.
   (2) **Microscopically,** multiple giant cells, resembling osteoclasts within a matrix of fibroblast-like cells with large, atypical nuclei, occur.

4. **Ewing's sarcoma** is a malignant neoplasm of undifferentiated cells arising within the marrow cavity. It is rare, usually affecting adolescents. Men are affected more often than women.
   a. **Etiology.** The tumor arises from mesenchymal cells that have been shown to have some expression of neural antigens.
   b. **Clinical features** are pain, tenderness and early widespread dissemination.
   c. **Pathology**
      (1) **Grossly,** the tumor commonly affects the pelvis and metaphyses of long tubular bones. Cells erode through the cortex and invade surrounding tissues. Half of the cases have "onion skin" or concentric layering of new bone.
      (2) **Microscopically,** undifferentiated small cells resembling lymphocytes occur. Surface antigens make the diagnosis.

JOINTS

A. **Arthritis**

1. **Suppurative arthritis**
   a. **Pathogenesis.** The primary mechanism of suppurative arthritis is hematogenous seeding of joints during bacteremia, which is more common than direct invasion. Organisms include Gonococcus, Staphylococcus, Streptococcus, *H. influenzae,* and Gram-negative bacilli.
   b. **Clinical features** include tender, swollen, and erythematous joints that require rapid intervention to prevent permanent joint damage.
   c. **Pathology.** This disease is **usually monoarticular,** affecting a large joint. Characteristics of typical suppurative infection are **cloudy synovial fluid** with a **high neutrophil count** that clots readily. If the organism is very virulent or if it is left untreated, the synovium may ulcerate and infection may erode articular cartilage.

NOTE

Systemic lupus erythematosus typically includes joint pain. Because it is a multisystemic immune disorder, it is discussed in the Clinical Immunology section of the Microbiology/Immunology review book.

IN A NUTSHELL

Suppurative arthritis:

- *Manifested by a tender, red, swollen joint (e.g., "a hot knee").*
- *Usually monoarticular, high neutrophil count in joint fluid, and often due to Staph, Strep, and Gonococci.*

2. **Tuberculous arthritis**
   a. **Incidence.** This form occurs more commonly in children.
   b. **Clinical features** include an insidious onset and joint destruction.
   c. **Pathology.** It occurs most often in the spine and hip. The synovial lining is covered with tubercles and granulation tissue. Pannus develops over the articular cartilage and may erode it. Destruction of joint space ensues with fibrosis and calcification, eventually leading to ankylosis.
3. **Osteoarthritis** (degenerative joint disease)
   a. **Incidence.** Osteoarthritis increases with age, affecting women more than men. It affects 80% of people over 70 years old in at least one joint.
   b. **Pathogenesis**
      (1) Aging or **wear and tear** (biomechanical) is the most important mechanism. Also, chondrocyte injury and abnormal collagen activity (biochemical) contribute; usually, both act together.
      (2) Predisposing factors include obesity, previous joint injury, and synovial disease. Most retired football players have at least some osteoarthritis in the knees and ankles.
   c. **Clinical features.** There is an insidious onset with joint stiffness, decreased range of motion, effusions, crepitus, and bony swelling. Symptoms of nerve compression may develop secondary to compression by osteophytes.
   d. **Pathology**
      (1) The most commonly affected joints include vertebrae, hips, knees, and distal interphalangeal (DIP) joints of fingers.
      (2) **Joint mice** are flakes of cartilage in the joint space from erosion.
      (3) **Osteophytes and bone spurs** develop. Denuded, sclerotic, subchondral bone may become exposed in areas (eburnation).
4. **Rheumatoid arthritis** is a systemic chronic inflammatory disease characterized by progressive arthritis. There are many clinical variants.
   a. **Incidence.** Women are affected three times more frequently than men. There is a familial predisposition, and the disease commonly presents from ages 20-60 years.
   b. **Pathogenesis** involves an **autoimmune reaction** with the formation of circulating antibodies **(rheumatoid factor)** against the Fc fragment of autologous IgG, leading to immune complexes.

IN A NUTSHELL

Osteoarthritis:

- *Due to wear and tear on joints.*
- *Erosion of articular cartilage leads to bone eburnation and chipping.*
- *X-rays show the loss of joint space.*
- *Heberden's nodes are found at the DIP joint.*

c. **Clinical features**
   (1) Symptoms include low-grade fever, malaise, fatigue, and **morning stiffness.**
   (2) Physical examination shows joint swelling, redness, and warmth. In late stages, ankylosis may develop.
   (3) Synovial fluid shows increased cells (usually neutrophils) and poor mucin.
   (4) There is an elevated sedimentation rate and hypergammaglobulinemia. The level of rheumatoid factor may correlate with the severity of the arthritis.
   (5) X-rays show erosions and osteoporosis.
   (6) Systemic features include subcutaneous nodules (20% patients), Sjögren's syndrome (15%), glaucoma, pericarditis, vasculitis, hepatosplenomegaly, and adenopathy.

d. **Pathology**
   (1) The disease usually starts in the **small joints of the hands and feet** but may involve any joint. There is **usually symmetric involvement.** Patients develop a diffuse proliferative synovitis in which the synovium becomes replaced by **pannus,** a vascularized mass packed with lymphocytes, macrophages, and plasma cells. Pannus erodes articular surfaces, bone, joint capsule, and ligaments. Adhesions and ankylosis may result.
   (2) Rheumatoid nodules are composed of proliferative connective tissue with areas of central necrosis. They may be seen in skin, heart valves, lung, pleura, pericardium, and spleen. Skin nodules are usually on extensor surfaces.
   (3) Arteries may show acute necrotizing vasculitis due to circulating antigen-antibody complexes.

5. **Gout.** In gout, there is **hyperuricemia** associated with recurrent bouts of acute arthritis, resulting from deposition of **monosodium urate in joint tissues.**
   a. **Types**
      (1) In **primary gout** (90% of cases), there is an **inborn error of purine metabolism.** The metabolic defect is usually not known. Specific enzyme defects account for only about 10% of cases (e.g., Lesch-Nyhan syndrome).
      (2) **Secondary gout** is hyperuricemia resulting from a disorder unrelated to purine metabolism (e.g., excessive cell breakdown as in leukemia and polycythemia).
   b. **Incidence.** Most cases are in men, but it occasionally affects postmenopausal women. It is familial (primary gout) in about 20% of cases.
   c. **Pathogenesis** is an overproduction of uric acid (two-thirds of primary gout cases) or underexcretion of uric acid (one-third of primary gout cases).

d. **Clinical features.** There is an asymptomatic period of hyperuricemia (> 7 mg/dl) followed by acute episodes of **joint pain and swelling.** After approximately 10 years of recurrent attacks, chronic disabilities ensue (i.e., decreased range of motion; joint deformities). Uric acid kidney stones develop in up to 25% of patients.

e. **Pathology.** Precipitation of urate crystals in joint fluid causes an acute **inflammatory synovitis** with synovial edema and leukocytic infiltrate. It usually affects the joints of the lower extremities, particularly of the **large toe.** Formation of **tophi** (urate deposits surrounded by inflammatory cells, including foreign body giant cells) is pathognomonic. Tophi may form in the helix of ear, bursae, ligaments, and kidney.

IN A NUTSHELL

Gout:

- *Gout is the deposition of urate crystals leading to acute, painful attacks of arthritis. The big toe is classically affected.*
- *Gout may result from overproduction or underexcretion of uric acid.*
- *Tophi are pathognomonic.*

B. **Tumors**

1. **Synoviosarcoma**
   a. **Incidence.** This is a rare tumor with a peak incidence in early adulthood, affecting boys and girls equally.
   b. **Clinical features.** These tumors form slow-growing, painless masses. They are very aggressive with early metastases to the lung and pleura.
2. **Malignant fibrous histiocytoma**
   a. **Incidence.** This is a relatively common soft tissue malignancy, affecting adult men more than women.
   b. **Pathology.** Tumors arise in soft tissue or bones. They are located in the lower extremities more often than in the upper extremities and in the abdominal cavity. **Microscopically,** tumors are pleomorphic, composed of fibroblasts, histiocytes, and tumor giant cells.

SKELETAL MUSCLE PATHOLOGY

A. **Clinical features.** Signs and physical findings of muscle disease include myotonia (continuous tonic contraction), weakness, muscle atrophy (wasting), fasciculations (twitching), and pseudohypertrophy. The symptoms and history of muscle disease include various combinations of the following:

1. Tripping, clumsiness (distal weakness especially)
2. Difficulty climbing stairs or rising from chairs (proximal weakness)
3. Family history, which includes three common patterns of inheritance: sex-linked (X-linked) inheritance, autosomal recessive inheritance, and autosomal dominant inheritance.

Diagnosis. Laboratory findings that may help differentiate muscle diseases include:

1. **Creatine kinase** is elevated in myositis and some dystrophies.
2. The **erythrocyte sedimentation rate (ESR)** may be elevated in myositis or any other inflammation.
3. **Serum potassium** may be abnormal in periodic paralysis and raised whenever there is cell necrosis.
4. **Pyruvate or lactate** may be abnormal in metabolic, particularly mitochondrial, muscle diseases.
5. **Urinary myoglobin** is elevated if there is acute muscle destruction (e.g., rhabdomyolysis).
6. **Electromyography (EMG) and peripheral nerve conduction velocities (NCVs)** may help differentiate neurogenic from myopathic disorders; in general, proximal weakness is often myopathic, while distal weakness is often neurogenic.
7. **Muscle biopsy** is often the definitive diagnostic procedure in myopathic disorders. Diagnosis is made by the presence or absence of certain histologic features or by staining with enzymatic stains.

C. **Neurogenic muscle atrophy** covers a large class of disorders secondary to loss of normal nerve supply; muscle is lost secondarily.

1. Features of denervated muscle include fiber shrinkage and angulation with nuclear pyknosis.
2. Initially, the histologic picture is of scattered atrophic fibers, but, since a nerve typically supplies many fibers, a picture of group fiber atrophy eventually becomes apparent.
3. Both type I and type II fibers show atrophy.
4. Intact neighboring axons may sprout and reinnervate fibers, which results in a single fiber type, regardless of the initial fiber type. This is known as "fiber-type grouping." Fibers become heterogeneously grouped together according to the "fiber type" (white or red) of the innervating neuron.
5. Ultimately, denervated muscle is replaced by connective and adipose tissue.
6. Polyarteritis nodosa (PAN) may show muscle vascular infiltration and infarction of muscle and nerve; PAN is particularly notorious for causing mononeuritis multiplex by infarction of multiple peripheral nerves, leading to neurogenic atrophy.

IN A NUTSHELL

Myasthenia gravis is an autoimmune disease in which antibodies are made against NMJ acetylcholine receptors. Clinically, it presents with muscle weakness that worsens with use. Ptosis is commonly seen.

D. **Myasthenia gravis** typically affects young women.

1. **Pathogenesis.** This is an autoimmune disorder caused by **IgG directed against the acetylcholine (ACh) receptor.**
2. **Clinical features** include fluctuating weakness. This weakness involves muscles supplied by cranial and peripheral nerves with no sensory abnormalities, and it is especially profound late in the day.
3. **Diagnosis** is established by a decremental response to repetitive electrical stimulation on EMG or by clinical improvement when a cholinesterase inhibitor (e.g., edrophonium) is administered. Muscle biopsy shows group atrophy and, occasionally, mononuclear cell infiltrates. Patients may have thymic abnormalities, including thymoma (10%-20%) or thymic hyperplasia (70%-80%). In these patients, thymectomy is often curative.

IN A NUTSHELL

The myositides are disorders that probably have an autoimmune etiology. They are characterized by muscle fascicle atrophy, edema, and necrosis.

E. **Myositides** include both polymyositis and dermatomyositis. Both polymyositis and dermatomyositis are classified as autoimmune or collagen vascular diseases.

1. **Incidence.** Polymyositis is more common in females.
2. **Pathogenesis.** These disorders are associated with autoantibodies, with a cell-mediated immune response, C2 deficiency, and HLA-DR3.
3. **Clinical features**
   a. Neck and proximal limb muscle weakness, dysphagia and muscle pain, tenderness and swelling are all common in polymyositis and in dermatomyositis.
   b. Dusky erythema of the skin with plaques over the joints or purple discoloration of the eyelids (**heliotrope rash**) is also a feature of dermatomyositis.
   c. Adults with dermatomyositis are at increased risk of having internal malignancies, particularly stomach, colon, lung, and breast.
   d. Laboratory studies show **elevated creatine kinase** and ESR. Creatinuria can result from breakdown of muscle proteins.
   e. Electromyographic abnormalities reflect abnormal muscle function.
4. **Pathology.** There is variation in fiber size, peripheral atrophy in fascicles, necrosis, myofiber vacuolation, edema, and perivascular mononuclear cell infiltration.

. **Muscular dystrophies** are a heterogeneous group of disorders that have a common feature of muscle degeneration. Dystrophic muscles are shrunken, flabby, and pale. **Microscopically,** there may be vacuolization, cytoplasmic fragmentation, hyalinization, and necrosis.

1. **Duchenne's muscular dystrophy** is the most severe type of muscular dystrophy.
   a. **Pathogenesis.** This disorder shows **X-linked recessive** inheritance. The **dystrophin** protein, encoded on the X chromosome and normally present in muscle cell membranes, is usually absent.
   b. **Clinical features**
      (1) Elevation of creatine kinase and histologic degeneration precedes clinical features.
      (2) The classic presentation is with **pelvic girdle weakness and ataxia.** The course is progressive, and children are unable to walk by the age of 10.
      (3) **Pseudohypertrophy of the calves** is characteristic.
      (4) Myocardial muscle involvement accompanies other muscle degeneration and may cause death.
      (5) Mental retardation is also an associated feature.
2. **Becker's muscular dystrophy**
   a. **Pathogenesis.** This disease shows both X-linked recessive inheritance or spontaneous mutations in the same gene as Duchenne's dystrophy.
   b. **Clinical features.** Patients may walk until age 20 or 25. Cardiac lesions are mild or absent.
3. **Facioscapulohumeral muscular dystrophy (FMD)**
   a. **Pathogenesis.** Inheritance is **autosomal dominant,** but spontaneous mutation is common.
4. **Limb-girdle muscular dystrophy (LGD)**
   a. **Pathogenesis.** Inheritance is autosomal recessive.
5. **Myotonic dystrophy**
   a. **Pathogenesis.** This disorder arises with an autosomal dominant pattern or through spontaneous mutations.
   b. **Clinical features.** It is clinically unique from other dystrophies. Characteristics include weakness, atrophy, and myotonia (tonic contraction of an affected muscle with inability to voluntarily relax it). Head and neck muscles are frequently weak and atrophic, while limb involvement is usually distal (hands and feet). Patients may have cardiac arrhythmias, cataracts, frontal baldness, hypogonadism, and idiosyncratic reactions in anesthesia. Muscle enzymes and biopsies may be normal.

CLINICAL CORRELATE

Calf pseudohypertrophy (replacement of muscle tissue with adipose and fibrous tissue) is characteristic of Duchenne's muscular dystrophy.

NOTE

Becker's muscular dystrophy is essentially a less severe form of Duchenne's. In Becker's, dystrophin is usually present but is of abnormal size.

G. **Rhabdomyosarcoma** is a **malignant neoplasm** arising from striated muscle. It is the most common soft tissue sarcoma in children. Prognosis depends on the site of origin. Up to 40% have metastases at the time of diagnosis.

1. **Embryonal rhabdomyosarcoma**
   a. In infancy or childhood, it is most often located in head and neck tissues and is less aggressive than other forms.
   b. **Sarcoma botryoids** is an embryonal rhabdomyosarcoma with a **grape-like, soft, polypoid gross appearance.** It is usually located in the genitourinary, upper respiratory, or biliary tract. It is extremely aggressive.

H. **Skeletal muscle abnormalities in AIDS**

1. **Incidence.** Up to 50% of HIV-positive individuals have some form of muscular abnormality.
2. **The most common finding** is similar to polymyositis (i.e., inflammatory infiltrates with macrophages and muscle fibers showing coagulative or segmental necrosis). The muscle is not directly HIV-positive.
3. **Zidovudine (AZT)-induced myopathy** is characterized by multinucleated cells associated with myositis and by focal infection.

IN A NUTSHELL

A myositis-like syndrome may occur in AIDS as a result of infection or AZT treatment.

SKIN

A. **Epidermal lesions**

1. **Seborrheic keratoses** are **benign neoplasms** that usually arise in areas exposed to the sun. They are very common in late adulthood.
   a. **Clinical features.** Although they are usually left untreated, they may be removed if they become irritated, or for cosmetic purposes. Sudden development of multiple lesions may follow an inflammatory dermatitis, hormonal therapy, or may accompany an underlying malignancy.
   b. **Pathology**
      (1) **Grossly**, lesions are typically located on the face, back, or trunk. They are typically brown to **gray, scaly,** and **greasy.**
      (2) **Microscopically,** seborrheic keratosis is a squamoproliferative disorder characterized by hyperkeratosis, papillary epidermal hyperplasia, and occasionally, development of pseudo horn cysts (epidermal pseudocysts filled with keratin).
2. **Keratoacanthoma** is also a benign squamous lesion, arising in sun-exposed areas. It is most common in middle age.

NOTE

Scleroderma presents with sclerosis of the skin. It is discussed in detail in the Clinical Immunology section of the Microbiology/Immunology review book.

IN A NUTSHELL

Seborrheic keratoses are gray, scaly, greasy lesions with hyperkeratosis, epidermal papillary hyperplasia, and occasional keratin pseudocyst formation. They are benign.

a. **Clinical features.** Keratoacanthoma is a rapidly growing papule that must be distinguished from squamous cell carcinoma.

b. **Pathology**

(1) **Grossly,** lesions are located on the head and arms. They start as a round pink papule that grows within weeks up to 2 cm with a central depression filled with keratin.

(2) **Microscopically,** the squamous cells are well organized and not anaplastic, although mitoses are present during the rapid growth phase. A key feature of this neoplasm is a lip of normal, nondysplastic epidermis on both sides of the keratin-filled crater. Keratoacanthomas are said to be composed of large squamous cells with a hyaline, "ground-glass" cytoplasm.

3. **Fibroepithelial polyps** are benign. Also known as **skin tags,** these lesions are common in middle age but may also develop during pregnancy. They are also associated with diabetes or intestinal polyposis. They usually occur in intertriginous regions and on the neck. Skin tags are composed of benign squamous epithelium, covering a fibrovascular core.

4. **Basal cell carcinoma** is invasive, but it rarely metastasizes.

a. **Incidence.** It is most common in middle-aged or elderly individuals and those who have fair complexions. They occur on sun-exposed areas.

b. **Clinical features.** Basal cell carcinomas are locally aggressive and rarely metastasize. Complete excision is usually curative, but there is approximately a 50% recurrence rate from shave biopsies.

c. **Pathology**

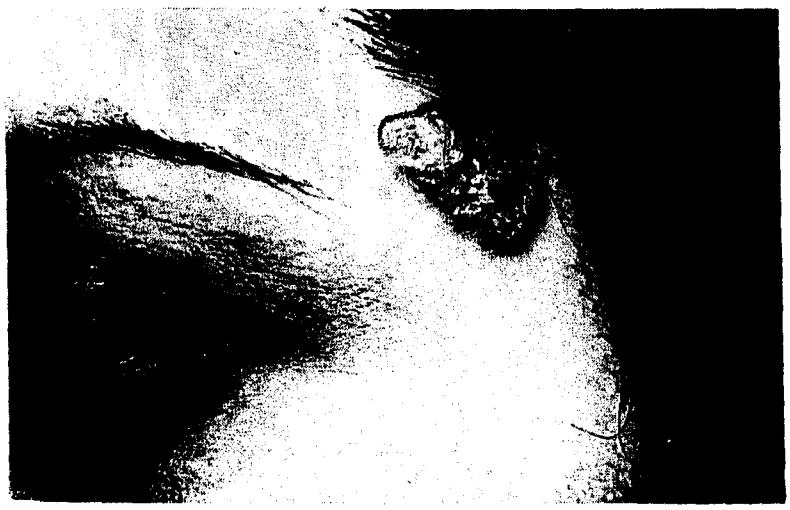


Figure 11-4. Basal cell carcinoma (gross).

CLINICAL CORRELATE

Skin tags are normal findings on most people; high numbers of them may indicate diabetes.

IN A NUTSHELL

Basal cell carcinoma:

- *Due to sun exposure*
- *Most commonly occur on the face*
- *Rare metastases*
- *Nests of tumor cells in desmoplastic tissue*
- *Pallisading of cells is characteristic*

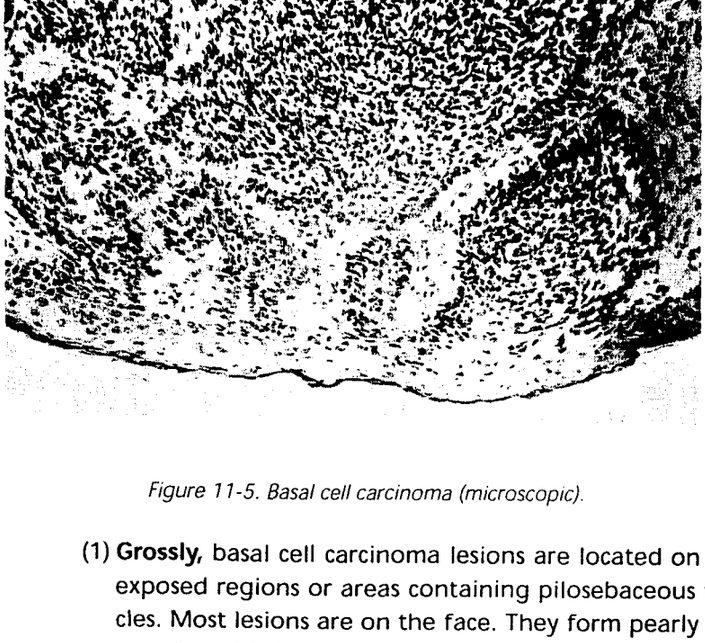


Figure 11-5. Basal cell carcinoma (microscopic).

(1) **Grossly,** basal cell carcinoma lesions are located on sun-exposed regions or areas containing pilosebaceous follicles. Most lesions are on the face. They form pearly grey papules with heaped up borders and a central depression (Figure 11-4).

(2) **Microscopically,** there are nests of tumor cells surrounded by a fibrous stroma. Pallisading of tumor cells at the edges of nests is a particularly useful microscopic characteristic (Figure 11-5).

5. **Actinic keratoses** are premalignant and may develop into squamous cell carcinoma.
   a. **Incidence** is highest in fair-skinned people of middle age. It is associated with chronic sun exposure.
   b. **Pathology**
      (1) **Grossly**, there are rough, crusty, red papules up to 1 cm in diameter.
6. **Squamous cell carcinoma** is a **malignant tumor** that is also found most frequently in **sun-exposed areas.**
   a. **Incidence.** The tumor peaks at 60 years of age with a preponderance among women.
   b. **Etiology.** Chronic sun exposure and fair complexion are the greatest risk factors. Chronic skin ulcers or sinus tracts, longterm exposure to hydrocarbons, burns, and radiation also contribute to risk.
   c. **Clinical features.** When squamous carcinoma occurs on sun-exposed regions, it rarely metastasizes. When it occurs on nonexposed skin, up to 50% metastasize, indicating a fundamentally different biology in two systems.

Figure 11-6. Squamous cell carcinoma (gross).

d. **Pathology**

(1) **Grossly,** the appearance is variable, depending on location and invasiveness. Squamous carcinomas may be firm, erythematous, scaly nodules or oozing ulcers with raised borders. On mucosal surfaces, they may be associated with leukoplakia (white plaques), made white by the keratin produced (Figure 11-6).

(2) **Microscopic findings** include atypical cells restricted to the epidermis (Bowen's disease or squamous cell carcinoma *in situ*) and atypical keratinocytes invading the dermis (invasive cancer). Atypical keratinocytes may form squamous pearls, i.e., laminated squamous cells with central keratinization in an "onion skin" configuration (Figure 11-7).

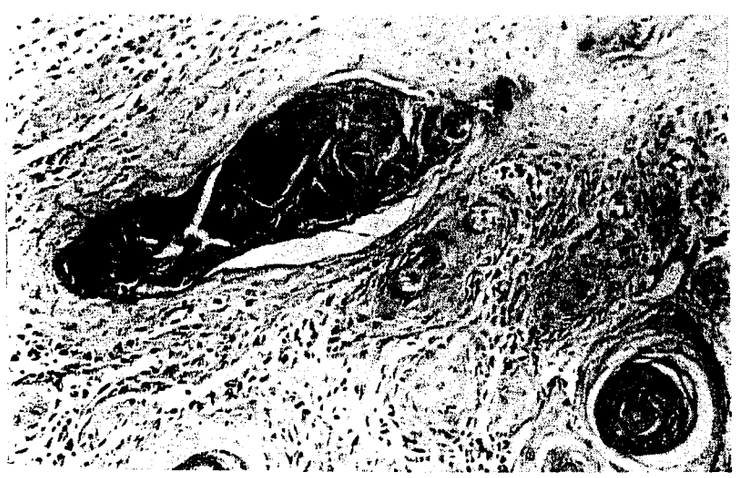


Figure 11-7. Well-differentiated squamous cell carcinoma with keratin pearls (microscopic).

IN A NUTSHELL

- *Squamous cell carcinoma arises in sun-exposed areas, skin ulcers, or sinus tracts.*
- *Grossly, squamous cell carcinoma may appear in many forms. Microscopically, "squamous pearls" (formed by atypical keratinocytes) help make the diagnosis.*

IN A NUTSHELL

Xanthomas are collections of lipid-laden histiocytes and are often associated with hyperlipidemia.

B. **Dermal lesions**

1. **Xanthomas**
   a. **Incidence.** Xanthomas may be idiopathic, or they may be associated with hyperlipidemia or malignancies.
   b. **Pathology.** They are **yellow nodules,** composed of foamy histiocytes with eosinophilic cytoplasm. The cells contain cholesterol, triglycerides, and phospholipids.
2. **Capillary hemangiomas** (strawberry hemangiomas)
   a. **Clinical features.** These lesions usually arise within the first weeks of life and usually resolve spontaneously, starting at 1-3 years of age; most are completely gone by age 5.
   b. **Pathology.** Capillary hemangiomas form a soft, red, lobulated mass, 1-6 cm in diameter, composed of thick-walled capillaries.
3. **Nevus flammeus** (port wine stain) is a common congenital lesion, composed of telangiectatic vessels. Usually located on the neck or face, it appears as a large, flat irregular pink patch that tends to resolve spontaneously.
4. **Kaposi's sarcoma** is a malignant mesenchymal tumor (an angiosarcoma), characterized by an aggressive course in patients with AIDS and by a slower course in elderly men.

IN A NUTSHELL

Kaposi's sarcoma is an angiosarcoma found on skin and mucous membranes. It is associated with AIDS.

C. **Pigmentary disorders**

1. **Freckles** are areas of increased melanin deposition in the basal cell layer of the epidermis.
2. **Vitiligo** is irregular, completely **depigmented patches.**
   a. **Incidence** is common and may affect any race. Risk is increased with a positive family history.
   b. **Etiology** is unknown, but it is possibly autoimmune or related to stress.
   c. **Pathology. Microscopically,** the skin is devoid of melanocytes in affected areas.
3. **Melasma** is irregular patches of hyperpigmentation on the face. It most commonly appears during pregnancy and does not completely regress.

IN A NUTSHELL

Vitiligo is characterized by irregular patchy depigmentation of unknown origin due to melanocyte deficiency.

D. **Melanocyte tumors**

1. **Nevocellular nevus** is a benign tumor of nevus cells and melanocytes.
   a. **Types of common nevi** include junctional, compound, and intradermal. Although the different types may have distinguishing clinical features, histologic examination is needed for accurate diagnosis.

b. **Clinical features**

(1) The relationship between nevi and melanoma is largely unknown except that both are clearly related to sun exposure. Although malignant transformation of nevi is not common, approximately 30% of cases of melanoma are associated with nevi. There is also increased incidence of melanoma associated with giant congenital pigmented nevi.

(2) In most cases, one can distinguish a benign nevus from melanoma on clinical grounds (i.e., color, contour). A nevus is tan to brown and has sharp, well-circumscribed borders. Color is usually uniform, and the lesions are stable in shape and size.

2. **Lentigo maligna** (Hutchinson's freckle)
   a. This is a premalignant lesion, occurring on sun-exposed surfaces in the elderly.
   b. It is characterized by intraepidermal proliferation of atypical melanocytes. Up to 50% progress to invasive melanoma over the course of several years.

3. ***Malignant melanoma***
   a. **Incidence.** Melanoma peaks by ages 40-60.
   b. **Pathology** (Figures 5-8 and 5-9)
      (1) **Lentigo maligna melanoma** arises from lentigo maligna with a peak incidence at age 70. This form of melanoma has the best prognosis.
      (2) **Superficial spreading melanoma** shows extensive horizontal growth with the radiating cells more atypical than those of lentigo maligna. Lesions are most commonly on legs, chest, and back; peak incidence is by age 60.

Figure 11-8. Melanoma (gross).

NOTE

Melanomas tend to grow horizontally before spreading vertically. Prognosis relates to depth of invasion.

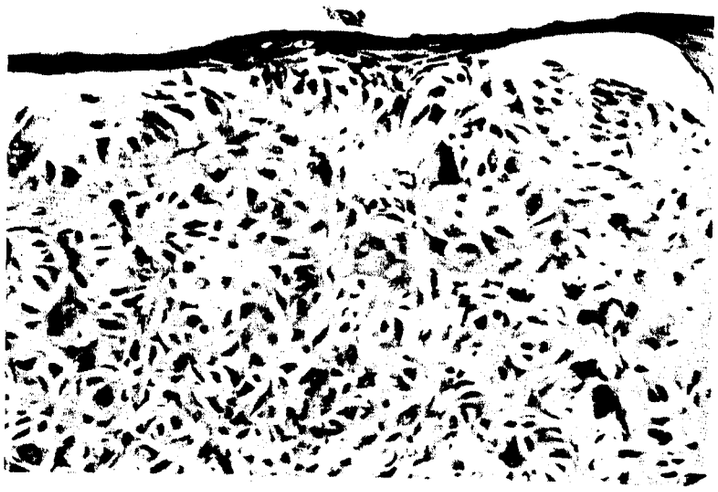


Figure 11-9. Melanoma (microscopic).

(3) **Nodular melanoma** shows extensive dermal invasion and rapid growth. Raised brown-black lesions may be found anywhere on the skin or mucosa. Peak incidence is by age 50 and has the worst prognosis of the melanomas.

c. **Diagnosis.** Staging is by **depth of invasion,** through the layers of the epidermis and dermis. Five-year survival rates range from 10% for the deepest invasion to 100% for the most superficial invasion.

d. **Treatment** is **complete excision.** Systemic disease is treated with chemotherapy or immunotherapy with poor but variable results. Some metastic melanomas resolve spontaneously, and some relapse as internal metastases more than a decade after a seeming "cure."

E. **Primary bullous disease**

1. **Bullous pemphigoid**

a. **Incidence.** This disorder is uncommon; however, it occurs more frequently than other primary bullous disease and tends to occur after age 60.

b. **Clinical features.** Bullous pemphigoid causes large, tense, pruritic bullae, usually on the lower abdomen, groin, inner thighs, and mouth. Most patients have **circulating autoantibodies against the dermoepidermal junction.** The disease follows a chronic relapsing course and is self-limited.

2. **Pemphigus vulgaris**

a. **Incidence** is most common from ages 40-60.

b. **Pathogenesis. Autoantibodies against the intercellular junctions between keratinocytes** cause acantholysis. **The**

loss of intercellular connections causes an altered cell configuration.

c. **Clinical features**

(1) Pemphigus starts with small vesicles, usually on the oral or nasal mucosa, then spreads to other parts of the body. Bullae are delicate and flaccid.

(2) **Nikolsky's sign** is the development of bullae, caused by rubbing the skin with a finger. Pemphigus may result in erosions; secondary infections may lead to 40% mortality. Lesions are treated with corticosteroids.

F. **Infectious diseases**

1. **Impetigo** is a superficial skin infection, usually caused by **Group A β-hemolytic Streptococci** or **Staphylococcus**. It is characterized by eroded pustules, covered by honey-colored crusts. Impetigo may lead to poststreptococcal glomerulonephritis.
2. **Molluscum contagiosum** is a **poxvirus** infection, causing development of multiple, small, firm, umbilicated papules with a characteristic microscopic appearance in which viral clusters cause eosinophilic inclusions in keratinocytes.
3. **Verrucae.** Warts are caused by **papillomaviruses,** which cause epidermal hyperplasia in a characteristic papillary configuration with hyperkeratosis and parakeratosis.
4. **Superficial fungal infections** may be caused by Trichophyton, Microsporum, and Malassezia. Infection is limited to the cornified layer of the epidermis.
   a. **Tinea capitis** ("cradle cap") affects the scalp in children.
   b. **Tinea corporis** infests the trunk and extremities of children. It usually presents as expanding round lesions with erythematous circinate borders.
   c. **Tinea vesicolor** causes hypo- or hyperpigmented groups of macules.
   d. **Tinea pedis** causes "athlete's foot."
   e. **Tinea cruris** causes "jock itch."
   f. **Tinea unguium** (onychomycosis) causes thickening and discoloration of the nail bed.
5. **Scalded skin syndrome** is a pediatric condition caused by an exfoliative toxin produced by *S. aureus*. The toxin splits the epidermis at the level of the stratum granulosum, causing a global denudation of the skin.

G. **Hypersensitivity reactions**

1. **Urticaria,** or hives, are usually transient, raised, pruritic, pink wheals, characterized by dermal edema.

NOTE

Tinea corporis is also known as "ringworm" and is actually a fungal infection.

2. **Eczema** is a class of very common, pruritic skin disorders, characterized by distinctive clinical and pathologic features.
   a. **Clinical forms**
      (1) **Atopic dermatitis** is of variable and often unknown etiology; usually, there is a family history of atopy (allergy).
      (2) **Contact dermatitis** may result from allergic or irritant exposure.
      (3) **Lichen simplex chronicus** causes chronic, lichenified plaques, probably caused by rubbing.
      (4) **Polymorphous light eruption** is seen after ultraviolet light exposure.
      (5) **Drug reactions** resolve when the offending drug is discontinued.
      (6) **Exfoliative dermatitis** describes scaling and erythema of the entire skin.
   b. **Pathologic types**
      (1) **Acute eczema** (i.e., contact dermatitis) describes edematous, oozing, red plaques, often with vesicles and dermal inflammation.
      (2) **Subacute eczema** (i.e., childhood atopic dermatitis) is associated with moist, red papules and plaques with epidermal hyperplasia and dermal inflammation.
      (3) **Chronic eczema**. Dry, scaly plaques are present for months. Lichenification causes accentuated skin creases and thickened skin.
   c. **Treatment.** Moisturizers can be used to control the itching; oral antihistamines and topical steroids may also be used.
3. **Erythema multiforme**
   a. **Pathogenesis.** This may be a hypersensitivity response to drugs (e.g., sulfonamides, penicillins), infections (e.g., herpes, mycoplasma), collagen vascular diseases, or malignancies.
   b. **Clinical features.** Erythema multiforme is uncommon. There is often symmetrical involvement of the limbs.
      (1) In the minor form, there are few lesions, no systemic symptoms; and the disease is self-limited.
      (2) In the major form **(Stevens-Johnson syndrome),** there is fever, respiratory difficulty, widespread skin involvement (including mucous membranes), a high risk of sepsis, and a risk of fatality.
   c. **Pathology.** A large erythematous papule that develops central vesiculation; erosion is classic. Lesions are also characterized by edema and inflammatory infiltration.

IN A NUTSHELL

Erythema multiforme is a hypersensitivity reaction to drugs. Stevens-Johnson syndrome is the severe form.

I. **Psoriasis**

1. **Incidence.** One percent of the population of the United States is affected. The peak incidence is 30 years of age, and the most common form is **psoriasis vulgaris.**
2. **Pathogenesis.** The etiology is unknown, but there is a clear genetic component. Precipitants include hormonal changes, infection, and trauma. Psoriasis may also be associated with arthritis, enteropathy, and myopathy.
3. **Clinical features of psoriasis vulgaris**
   a. Lesions are located throughout the body, especially on the nails, knees, elbows, and scalp. They usually do not involve mucous membranes.
   b. Lesions are well-demarcated coral-colored plaques with **white or silver scale.**
   c. The **Auspitz sign** is seen when removal of scale results in pinpoint areas of bleeding. This is characteristic of psoriasis.
4. **Pathologic features of psoriasis vulgaris**
   a. Hyperkeratinization with parakeratosis appears in a patchy distribution.
   b. Epidermal hyperplasia causes thickening and lengthening of the rete ridges, usually to a uniform depth.
   c. Thinning of the surface epidermis, particularly over the dermal papillae, is characteristic.
5. **Treatment** is usually with **topical steroids** and **ultraviolet irradiation.** Severe, systemic disease may be treated with methotrexate.

IN A NUTSHELL

Psoriasis:

- *A silvery, scaly plaque that primarily affects knees, elbows, and the scalp.*
- *Histologically, it is characterized by epidermal hyperplasia and hyperkeratinization.*

I. **Inflammatory disorders**

1. **Acne vulgaris** causes comedones, papules, and cysts. It may be related to hormones, drugs, diet, irritants, and genetic factors. An allergy to *Propionbacterium acnes* is clearly involved.
2. **Pityriasis rosea**
   a. **Incidence.** This disorder is common, from ages 10-40.
   b. **Pathogenesis.** There is a possible viral etiology.
   c. **Clinical features.** Pityriasis rosea presents first with a "Herald patch," an approximately 4-cm, red, scaling patch, followed within days by eruption in "turtle neck–short sleeve" distribution. Lesions are small, pink, oval patches along flexural lines (fir tree pattern), appearing in crops. The disease is usually self-limited (1-4 months).
3. **Rosacea**
   a. **Incidence.** Rosacea is common from ages 30-50. Women are affected three times more commonly than men, but the syndrome is more severe in men.

b. **Clinical features.** The lesions affect the central **face. Erythema** and **telangiectasias, acneform lesions** (i.e., papules, cysts, pustules), and **rhinophyma** (teleangiectasias and hyperplasia of nasal soft tissue) are all seen in various combinations, sometimes causing a severe distortion of the face, particularly the nose.

PART III

BIOCHEMISTRY

Acid-Base Equilibrium and Buffering

Homeostasis requires that the pH of biological fluids be maintained at a constant and characteristic value. The control of pH in body fluids and subcellular compartments is achieved by a number of biological buffers. The most important buffers in physiological systems are bicarbonate, phosphate, and proteins. Buffers consist of a weak acid and its conjugate base that are in equilibrium and are able to donate and accept protons from the surrounding medium. The relationship between pH, pK_a, and the concentration of the conjugate acid and base of a buffering system is described by the Henderson-Hasselbach equation. Disturbances in the acid-base balance can lead to acidosis (a plasma pH less than 7.35), or alkalosis (a plasma pH greater than 7.45). This chapter reviews the concepts underlying acid-base equilibrium, the pH of various body fluids, and the properties of the buffer systems that resist fluctuations in pH.

ACID-BASE EQUILIBRIUM

The most appropriate biochemical definition for acids and bases is that acids are proton donors and bases are proton acceptors. Many important compounds found in biological fluids, including amino acids, lactic acid, and acetoacetic acid, are weak acids.

A. **Properties of weak acids.** Weak acids, in contrast to strong acids such as HCl or H_2SO_4, are not completely dissociated under the conditions that exist within biological systems. The undissociated form (HA) that retains the proton is known as the **conjugate acid**, while the corresponding deprotonated form (A^-) is a **conjugate base** that can accept a proton to recreate the acid. The reaction that describes the dissociation of a weak acid is shown in Figure 1-1.

$$HA \rightleftharpoons H^+ + A^-$$

conjugate acid — conjugate base

Figure 1-1. Dissociation of a weak acid.

B. The **Henderson-Hasselbalch equation** shown in Figure 1-2 describes the relationship between pH and the concentration of a weak acid and its conjugate base. In this equation, pH is defined as the negative log of $[H^+]$ and pK_a is defined as the negative log of the dissociation constant K_a, where $K_a = \frac{[H^+][H^-]}{[HA]}$. For any weak acid, the pK_a is a constant that is characteristic of that particular ionizing group. The Henderson-Hassselbach equation is useful because it provides a meaningful definition of pK_a and it permits calculation of the amounts of the undissociated acid and its conjugated base at any pH.

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

Figure 1-2. Henderson-Hasselbalch equation.

NOTE

A corollary of the equation: When the pH is greater than the pK_a, the base (A^-) form is dominant; when the pH is less than the pK_a, the acid (HA) form is dominant.

1. **A functional definition of pK_a** can be deduced from the Henderson-Hasselbalch equation. The pK_a of any ionizing group can be defined as the pH where the concentrations of the acid (HA) and its conjugate base (A^-) are equal. Conversely, when the pH is equal to the pK_a of the acid, the concentrations of HA and A^- are equal. Inspection of the equation indicates that when $[HA] = [A^-]$, the last term of the equation becomes zero and the equation reduces to $pH = pK_a$.
2. The logarithmic nature of the Henderson-Hasselbalch equation indicates that small **changes in pH** will bring about large changes in the relative concentrations of HA and A^-. These effects are summarized in Table 1-1, where lactic acid, having a pK_a of 4.8, is considered as an example of a weak acid. The same principles apply for any weak acid, i.e., a change of 1 pH unit results in a 10-fold change in the ratio of conjugate acid to conjugate base.

	pH	[lactic acid]/[lactate$^-$]
	2.8	100
	3.8	10
pK_a	4.8	1
	5.8	0.1
	6.8	0.01

Table 1-1. Effect of pH on the relative amounts of lactic acid and lactate.

TITRATION AND BUFFERING PROPERTIES OF WEAK ACIDS

Titration can be defined as the addition of a strong base to a weak acid (or a strong acid to a weak base) for the purpose of buffering the solution, i.e., maintaining a constant pH.

A. **Titration curve**. The addition of a strong base such as sodium hydroxide to a weak acid may be written as:

$$HA + NaOH \longrightarrow NaA + H_2O$$

As NaOH is added, the protons released by the weak acid combine with hydroxide to form water. The continued addition of sodium hydroxide progressively decreases the $[H^+]$ and increases the pH. A graph of the titration of a weak acid with a strong base is shown in Figure 1-3.

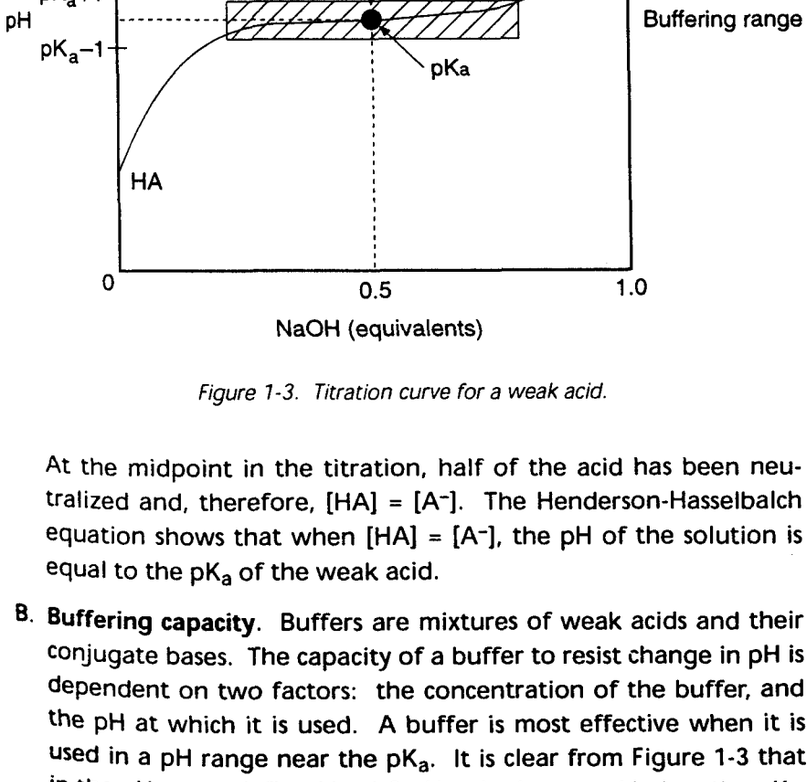


Figure 1-3. Titration curve for a weak acid.

At the midpoint in the titration, half of the acid has been neutralized and, therefore, $[HA] = [A^-]$. The Henderson-Hasselbalch equation shows that when $[HA] = [A^-]$, the pH of the solution is equal to the pK_a of the weak acid.

B. **Buffering capacity**. Buffers are mixtures of weak acids and their conjugate bases. The capacity of a buffer to resist change in pH is dependent on two factors: the concentration of the buffer, and the pH at which it is used. A buffer is most effective when it is used in a pH range near the pK_a. It is clear from Figure 1-3 that in the pH range defined by 1.0 pH unit above and below the pK_a,

NOTE

The best buffering occurs when pK_a = pH, resulting in 50% of acid and 50% of base. This system can then handle either an addition of base or an addition of acid.

the addition of either strong acid or strong base will result in minimal change in the pH.

PHYSIOLOGICALLY IMPORTANT BUFFERING SYSTEMS

The three most important buffering systems in biological systems are proteins, bicarbonate, and phosphate. The conjugate acid-base pairs and the pKa values for these buffers are summarized in Table 1-2. The major buffer in plasma and interstitial fluid is bicarbonate, whereas protein and organic phosphate esters are the major buffers of intracellular fluid.

A. **Protein buffering systems.** The cytosol of cells contains high concentrations of proteins with amino acid side chains that are weak acids and bases. These side chains impart great buffering capacity to proteins. The acidic amino acids (glutamic and aspartic acids) and the basic amino acids (lysine, histidine, and arginine) all contain ionizable side chains (discussed in the next chapter). However, histidine is the only amino acid with good buffering capacity at physiological pH. The **imidazole side chain of histidine** has a pK_a that ranges from 5.6-7.0, depending on its microenvironment within the protein.

BRIDGE TO PHYSIOLOGY

Histidine is frequently found in the catalytic site of enzymes where the imidazole ring can donate or accept protons in the formation and breaking of bonds.

Buffering system	pK_a
Protein systems:	
Histidine side chains	5.6-7.0
Bicarbonate system:	
HCO_3^-/CO_2	6.1
Phosphate systems:	
$HPO_4^{2-}/H_2PO_4^-$	6.8
Organic phosphate esters	6.5-7.5

Table 1-2. Properties of major physiological buffers.

The buffering reaction for proteins is shown in Figure 1-4, in which the imidazole side chain of histidine can reversibly donate and accept protons. The presence of multiple histidine side chains within each protein molecule contributes to the buffering capacity of the protein. Histidine plays a key role in making hemoglobin an excellent buffer in red blood cells.

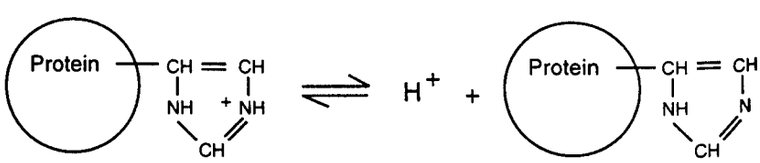


Figure 1-4. Protein buffering system.

B. **Bicarbonate buffering system**. The bicarbonate-CO_2 system is the most important buffer in **maintaining the pH of blood plasma and interstitial fluid** at its normal value of 7.4. Figure 1-5 summarizes the propeties of this buffering system. Carbonic acid (H_2CO_3) is the proton donor, bicarbonate anion (HCO_3^-) is the proton acceptor, and the pK_a for this reaction is 6.1. The strength of this buffering system lies in the ability of carbonic acid to be converted to carbon dioxide.

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \qquad pK_a = 6.1$$

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

Figure 1-5. The bicarbonate/CO_2 buffering system.

C. **Phosphate buffering system**. Intracellular fluids contain high concentrations of inorganic phosphate and many organic phosphate esters that contribute significantly to the buffering power of the cytosol. The phosphate buffering system is of little importance in plasma and interstitial fluid because of the low concentrations of phosphates in extracellular fluids. The phosphate buffering system **consists of $H_2PO_4^-$ as the proton donor and HPO_3^{2-} as the proton acceptor**. The pK_a of 6.8 is sufficiently close to the normal intracellular pH to make it an ideal buffer in those fluids that contain high concentrations of phosphates, such as red blood cells and kidney tubules.

BRIDGE TO PHYSIOLOGY

Acidosis, a plasma pH below 7.35, can result from the accumulation of acids such as lactic acid, acetoacetic acid, and β-hydroxybutyric acid (resulting in a metabolic acidosis), or from hypoventilation that results in the accumulation of CO_2 (respiratory acidosis). Alkalosis, a plasma pH above 7.45, can result from a loss of stomach acids due to excessive vomiting (metabolic alkalosis) or from hyperventilation (respiratory alkalosis).

Proteins, Enzymes, and Coenzymes

Proteins are linear polymers of amino acids, each having distinct chemical and structural properties. They are the largest class of molecules found in living systems, and are responsible for most of the diverse functions of the cell. They act as transport proteins, storage proteins, hormone receptors, structural proteins, regulatory proteins, and enzymes. Each protein has a unique amino acid sequence that determines its precise three-dimensional conformation and specifies its function. The most versatile class of the proteins are the enzymes, which act as biological catalysts, enhancing the rate of the chemical reactions occurring in cells. Most enzymes catalyze only one reaction and their catalytic activities may be stringently regulated, allowing the cell to control and coordinate its metabolic pathways. The kinetic parameters, K_m and V_{max}, are constants that describe the catalytic properties of enzymes. The function of many enzymes requires a cofactor—a metal or a small organic coenzyme derived from a vitamin precursor. This chapter will review the structure and properties of amino acids that are found in proteins, the kinetic and regulatory properties of enzymes, and the relationship between coenzymes and their vitamin precursors.

AMINO ACIDS: THE BUILDING BLOCKS OF PROTEINS

A. **Amino acid structure.** The building blocks for proteins are the 20 common amino acids that are encoded in the DNA of the cell.

1. **General structure.** Nineteen of the twenty common amino acids can be represented by the structure shown in Figure 2-1.

They all have a central carbon atom attached to a carboxyl group, an amino group, and a hydrogen atom. The amino acids differ from one another only in the chemical nature of the side chain (R). The only amino acid that is not described by this structure is proline, an imino acid in which the side chain forms a cyclic structure with the amino group.

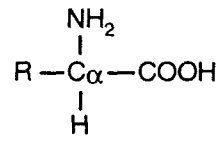


Figure 2-1. General structure of α-amino acids.

2. **Classification.** The amino acids can be classified as either hydrophobic or hydrophilic, depending on the ease with which their side chains interact with water. This particular method of classification is especially useful when considering amino acids as the building blocks of proteins. The properties of amino acid side chains influence how the protein folds into more compact structures. In general, proteins fold so that the hydrophobic side chains are in the interior of the molecule where they are protected from water and the hydrophilic side chains are on the surface.
   a. **Hydrophobic** amino acids have side chains with aliphatic groups or aromatic ring structures (Figure 2-2).
   b. **Hydrophilic** amino acids have side chains that contain O, N, or S atoms. Some of the hydrophilic side chains are charged at physiological pH. The acidic amino acids (aspartic and glutamic acids) have carboxyl groups that are negatively charged, while the basic amino acids (lysine, arginine, and histidine) have nitrogen atoms that are positively charged. Other hydrophilic amino acids with uncharged side chains contain hydroxyl groups (serine and threonine), a sulfur atom (cysteine), or amide groups (asparagine and glutamine). The structures of the R groups for the hydrophilic amino acids are shown in Figure 2-3.

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Hydrophilic amino acid side chains

Positive	*Negative*	*Neutral*
Lys	*Asp*	*Ser*
Arg	*Glu*	*Thr*
His		*Cys*
		Met
		Asn
		Gln

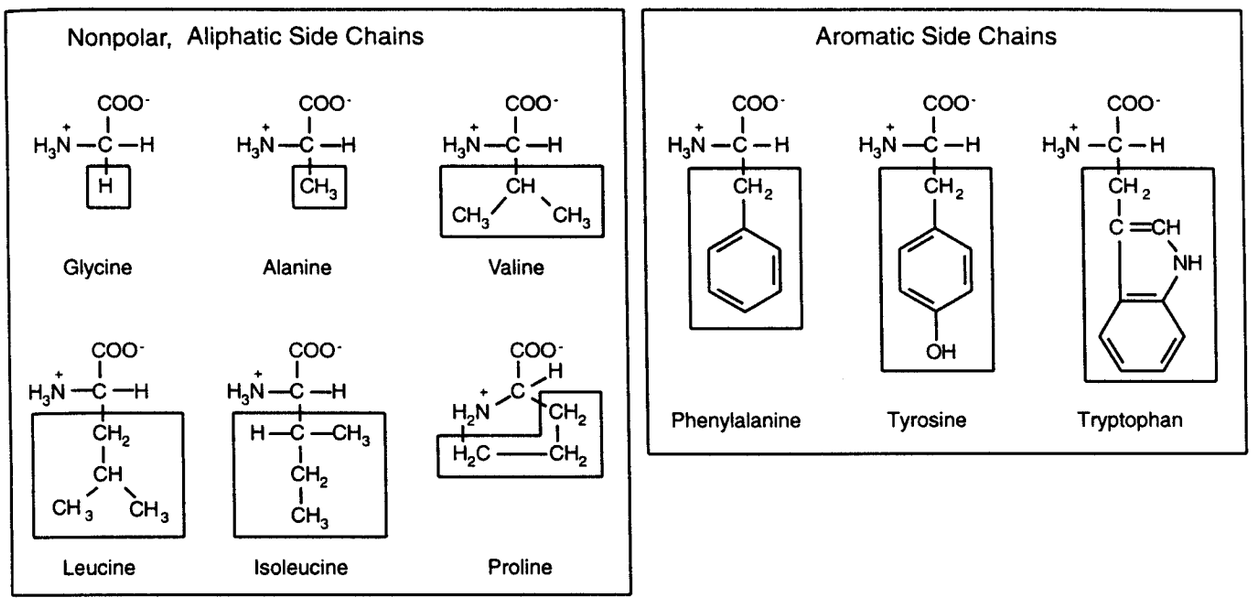

Figure 2-2. The hydrophobic amino acids.

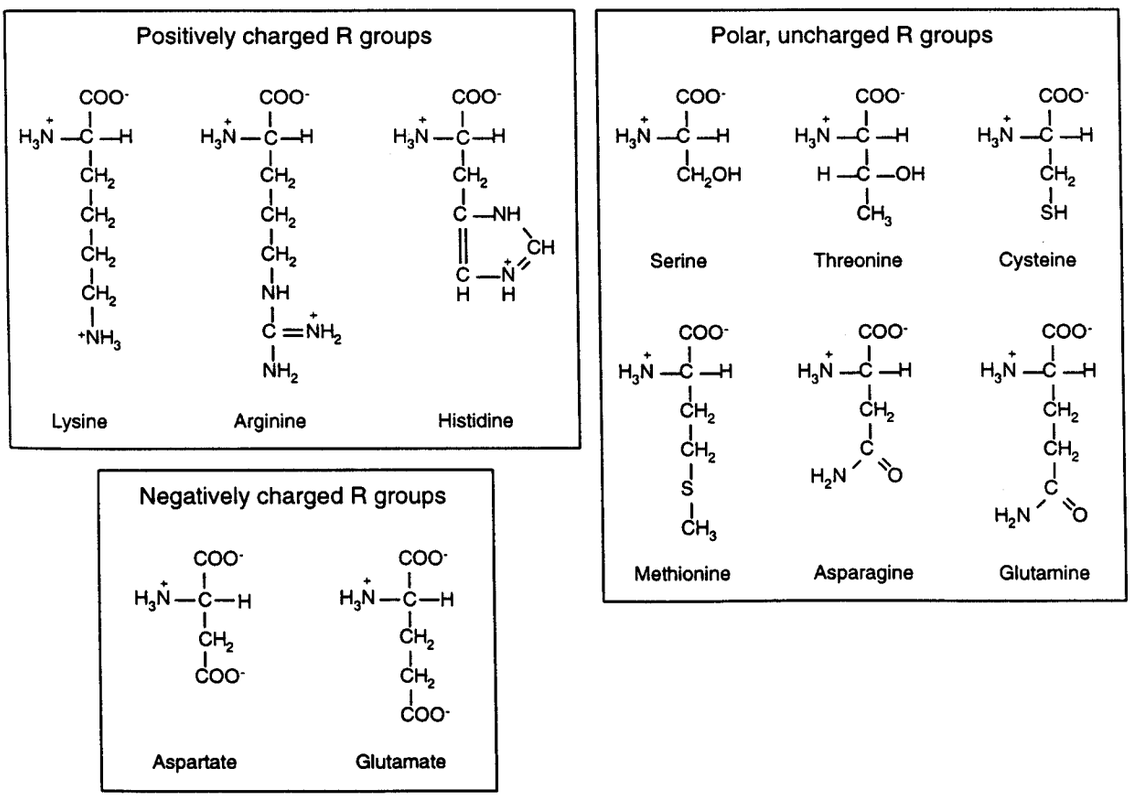

Figure 2-3. The hydrophilic amino acids.

3. **Modified amino acids.** In addition to the 20 common amino acids that have their origin in the genetic code, proteins often contain other amino acids that are generated by modification of the common amino acids after the protein has been synthesized. This process is called **post-translational modification.**
   a. **Cystine** is formed in proteins by the reaction of two cysteine side chains to form a disulfide linkage. It is found most frequently in extracellular proteins where the disulfide bond stabilizes the three-dimensional structure of the protein. This is the only covalent cross-linkage found in proteins.
   b. **Hydroxyproline** is formed in an oxygen-dependent hydroxylation reaction that occurs in fibroblasts. It is found in collagen, where it stabilizes the triple helical structure.
   c. **Phosphotyrosine, phosphoserine,** and **phosphothreonine** are formed by transferring phosphate from ATP to the hydroxyl group of serine, tyrosine, or threonine. These amino acids are found in many enzymes and proteins, where they serve as regulatory signals.

B. **Properties of amino acids.** The properties of proteins are influenced by the properties of their constituent amino acids. In particular, the stereochemistry and ionic properties of amino acids have an impact on the structure and/or properties of the protein.

1. **Stereochemistry.** The α-carbon atom of all amino acids except glycine is linked to four different chemical groups, making the α-carbon atom an asymmetric center. As shown in Figure 2-4, an asymmetric center has two stereoisomers (enantiomers) that are mirror images of each other and are designated as D- and L-amino acids. Only L-amino acids are incorporated into proteins.

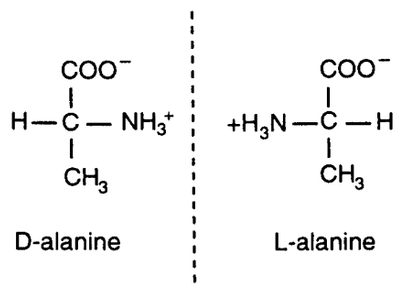

Figure 2-4. Stereoisomers of an α-amino acid.

2. **Ionic properties.** Depending on the pH, an amino acid may have no net charge, or it may have either a positive or a negative charge. The pH at which a molecule is electrically neutral

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- *S-S bridges:* *Cystine*
- *Collagen triple helix:* *Hydroxyproline*
- *Signal transduction:* *Phosphotyrosine, Phosphoserine, Phosphothreonine*

NOTE

There are other amino acids like ornithine and citrulline that play important roles in the cell but that are not part of the building block of 20.

is defined as the **isoelectric point** (pI). The equilibrium between the ionic forms of an amino acid is shown in Figure 2-5. The pK_a values for the α-carboxyl and α-amino groups are approximately 2.1 and 9.8, respectively. At a pH below the pK_a, the protonated species predominates, whereas at a pH above the pK_a, the deprotonated species predominates. The pI for the amino acid shown in Figure 2-5 is the average of the two pK_a values. At neutral pH, the species that has both positive charge and negative charge is called a **zwitterion.**

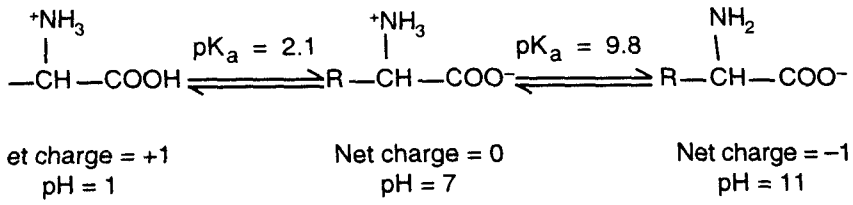

Figure 2-5. The ionic equilibrium for an α-amino acid.

The acidic and basic amino acids also have ionizing groups in their side chains. Aspartic and glutamic acids have side chain carboxyl groups with a pK_a of approximately 4.0, and are negatively charged at physiological pH. Lysine and arginine have side chains with protonated nitrogen atoms having pK_a values of approximately 10, and are positively charged at physiological pH. Histidine has an imidazole side chain with a pK_a of approximately 6.0, a value sufficiently close to the physiological pH to allow some of the histidine side chains to be positively charged while others have no charge. The ratio of positively charged histidine side chains to uncharged side chains at any pH can be calculated from the Henderson-Hasselbach equation (described in the previous chapter).

PROTEINS

Each protein has a unique three-dimensional structure dictated by its amino acid sequence and responsible for the highly specific function of the protein.

A. **Structural features.** The amino acids in a protein are linked together by peptide bonds in which the α-carboxyl of one amino acid is linked to the α-amino group of another amino acid (Figure 2-6). The peptide (amide) bond has partial double-bond character, making it planar and rigid, thereby imposing limitations on higher orders of structural organization in a protein.

1. **Primary structure.** The sequence in which the amino acids occur in the polypeptide is defined as the primary structure. This sequence is encoded by the DNA, and it determines how the protein folds into a more compact three-dimensional structure.

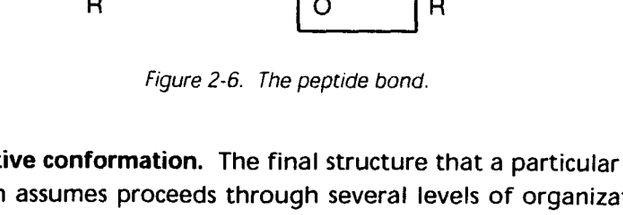

Figure 2-6. The peptide bond.

2. **Native conformation.** The final structure that a particular protein assumes proceeds through several levels of organization. In contrast to the primary structure, which was stabilized by strong covalent bonds, higher orders of structure are stabilized by numerous weak noncovalent interactions.
   a. **Secondary structure** in proteins is organized around the polypeptide backbone and is stabilized by large numbers of hydrogen bonds formed between the amide hydrogen atom of one peptide bond and the carbonyl oxygen atom of another. Proteins contain two major types of secondary structure: **α-helical structure**, which is stabilized by intrachain hydrogen bonds, and **β-sheet structure**, which is stabilized by interchain hydrogen bonds. In some fibrous proteins, the highest order of structure is secondary. These proteins are elongated asymmetrical proteins that frequently function as structural components of cells and tissues. Simple combinations of a few secondary structure elements are called **motifs.** An example of a motif would be a β-α-β motif that can be used to connect two parallel β strands. The α-helix in the middle of the two β strands allows for the reversal of direction.
   b. **Tertiary and quaternary structure.** Segments of secondary structure in globular proteins associate with one another and fold into a tertiary structure that is stabilized by noncovalent interactions between amino acid side chains. The fundamental unit of tertiary structure is the **domain,** a part of the polypeptide chain that folds into a stable tertiary structure. Proteins that are composed of more than one polypeptide chain have quaternary structure. Both tertiary and quaternary structures are stabilized by ionic and hydrophobic interactions and by hydrogen bonding between side chains.

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1° structure:	• *Amino acid sequence*
2° structure:	• *α-helix* • *β-sheet*
3° structure:	• *Overall protein structure* • *How α-helix and β-sheet fold with respect to each other*
4° structure:	• *Mutiple polypeptide chains* • *How chains fold with respect to each other*

3. **Denaturation** is defined as the loss of native conformation, resulting in a random coil that has little, if any, of the biological activity of the native protein. Denaturation may be caused by heat, extremes in pH, or by detergents.

B. **Methods for protein purification and structural analysis.** Before the structure of a protein can be studied, it must first be separated from other proteins in solution.

1. **Purification methods** are based on differences in the physical, chemical, and biological properties of a mixture of proteins in solution.
   a. **Electrophoresis** separates proteins on the basis of their charge/size ratio.
   b. **Gel filtration** chromatography and ultracentrifugation separate proteins on the basis of size.
   c. **Affinity chromatography** is based on the principle that different proteins have binding sites that are highly specific for certain small molecules. Immunoaffinity chromatography uses antibodies directed against specific segments of a protein's structure.
2. **Structural analysis.** Many methods exist for obtaining information about the structure of a protein.
   a. **Amino acid composition** requires hydrolysis of the protein into amino acids that are then separated from one another by chromatographic procedures and quantitated by reaction with a fluorescent reagent.
   b. **Sequencing** of a protein is achieved by Edman degradation. It begins at the N-terminal end of the protein, where the free α-amino group is reacted with phenylisothiocyanate (Edman reagent). Hydrolysis under anhydrous conditions releases the modified N-terminal residue, leaving the remainder of the peptide chain intact. The modified N-terminal residue is separated and identified chromatographically, and the residual peptide chain is carried through another cycle of degradation. The analysis of large proteins is facilitated by their cleavage into smaller peptide fragments, which can be analyzed separately. Trypsin, chymotrypsin, and cyanogen bromide are reagents used to cleave proteins. Since each of these reagents cleaves at different, but highly specific sites, the fragments can be aligned by looking for "overlapping sequences."
   c. **Optical rotary dispersion** and **circular dichroism** provide information about the secondary structure of a protein.
   d. **Ultraviolet spectroscopy** and **nuclear magnetic resonance** give detailed information about protein conformation in solution.

e. **X-ray crystallography** provides detailed information on the three-dimensional structure of the protein in the crystalline state.

C. **Function.** Proteins perform most of the important functions of the cell. Many of the proteins in skin, bone, muscle, and hair perform structural roles. Other proteins carry out the dynamic functions of the cell. Many plasma proteins transport small molecules such as iron or oxygen; immunoglobulins and clotting factors participate in defense functions; hormones, hormone receptors, and transcription factors play critical regulatory roles in the cell; enzymes function as biological catalysts.

ENZYMES

Virtually all biologically important reactions are catalyzed by enzymes. Most of these reactions occur under very mild conditions of temperature and pH that are compatible with living organisms. In the absence of enzymes, reactions in the cell would proceed at insignificant or undetectable rates. Enzymes differ from inorganic catalysts in their specificity, catalytic efficiency, and regulatory properties.

A. **Classification.** Enzymes are divided into six different classes on the basis of the types of reactions they catalyze (Table 2-1). All of the thousands of different reactions occurring in cells fall into one of these six types of reactions.

B. **Specificity.** The high-level specificity of enzyme-catalyzed reactions occurs because enzymes have active sites composed of a small number of amino acid side chains. The side chains come together to form a three-dimensional site on the surface of the

Class	Type of Reaction
Oxidoreductases	Oxidation-reduction reactions Frequently use coenzymes NAD^+, FAD, $NADP^+$, or O_2 as electron acceptors (Dehydrogenase, oxidase, reductase)
Transferases	Transfer of a chemical group from a donor to an acceptor Groups transferred include amino, carboxyl, acyl, glycosyl, phosphoryl (Transaminase, kinase)
Hydrolases	Cleavage of a bond between carbon and some other atom by the addition of water (Protease, phosphatase, amylase)
Lyases	Nonhydrolytic cleavage of carbon-carbon, carbon-sulfur and some carbon-nitrogen bonds (Aldolase, decarboxylase, dehydratase)
Isomerases	Interconversion of isomers (Epimerase, mutase)
Ligases	Formation of bonds between carbon and oxygen, nitrogen, or sulfur atoms in reactions that require energy (Carboxylase, thiokinase)

Table 2-1. The six classes of enzymes.

enzyme that is complementary to the structure of the substrate. Some of the amino acid side chains in the active site participate in binding the substrate to the enzyme; others act as catalytic groups and enhance the rate of the reaction by acting as acids, bases, or nucleophiles.

Catalytic properties. The energy profile of a chemical reaction, as it proceeds from substrate to product, is shown in Figure 2-7. The highest point on the curve is the energy of the **transition state,** an intermediate whose properties resemble both the substrate and the product. The energy barrier created by the transition state is also known as the **activation energy** ($\Delta G^{\ddagger}$) of the reaction. To initiate the reaction, energy must be expended to overcome the energy barrier. The rate of a reaction is inversely proportional to the magnitude of the activation energy. As shown by the dotted line in Figure 2-7, enzymes increase the rate of a reaction by decreasing the activation energy barrier. They have no effect on the equilibrium constant for the reaction, which is related to ΔG, the energy difference between the products and substrates.

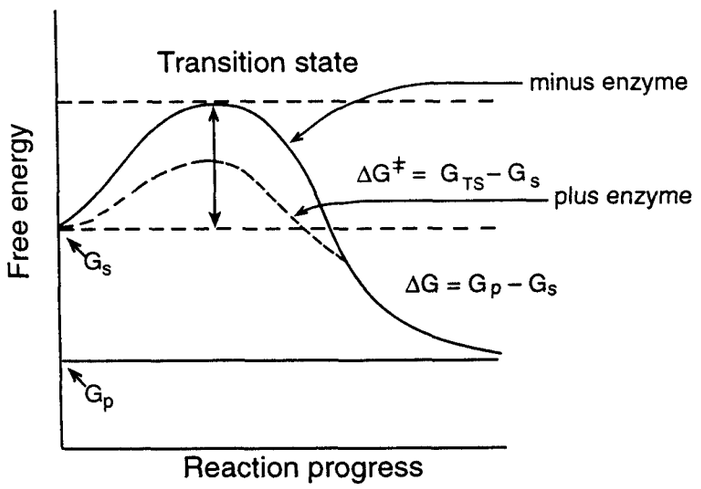

Figure 2-7. Energy profile for a catalyzed and uncatalyzed reaction.

D. **Kinetic properties of enzymes.** Kinetics is the study of the rate at which chemical reactions occur.

1. Factors affecting the rate of enzyme-catalyzed reactions include temperature, pH, enzyme concentration, and substrate concentration. Typical rate responses to these factors is shown in Figure 2-8.

a. **Temperature.** The rate of most reactions increases approximately two-fold with a 10° C increase in temperature. For enzyme-catalyzed reactions, however, there is an optimum temperature beyond which the rate rapidly decreases due to denaturation of the enzyme.

b. **pH.** The optimal activity of most enzymes occurs between pH 5 and 9. The shape of the rate-vs-pH curve reflects different ionization states for specific amino acid side chains that are required for substrate binding or for catalysis.

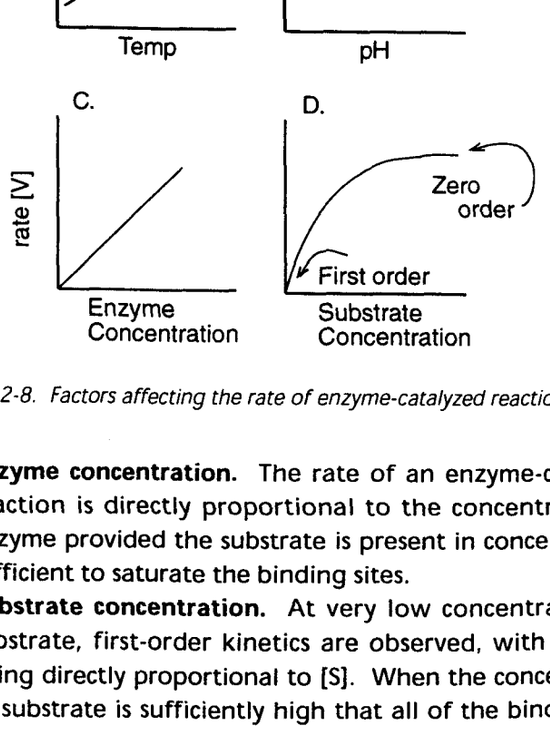

Figure 2-8. Factors affecting the rate of enzyme-catalyzed reactions.

MNEMONIC

STEP up the reaction rate:

***S**ubstrate concentration*
***T**emperature*
***E**nzyme concentration*
***p**H*

c. **Enzyme concentration.** The rate of an enzyme-catalyzed reaction is directly proportional to the concentration of enzyme provided the substrate is present in concentrations sufficient to saturate the binding sites.

d. **Substrate concentration.** At very low concentrations of substrate, first-order kinetics are observed, with the rate being directly proportional to [S]. When the concentration of substrate is sufficiently high that all of the binding sites are occupied, zero-order kinetics are seen, with the rate being independent of [S].

2. The **Michaelis-Menten equation** is an expression that quantifies the relationship between the rate of an enzyme-catalyzed reaction and the substrate concentration. In deriving an equation for the curve shown in Figure 2-8 (D), it is assumed that the limiting rate observed at an infinitely high substrate concentration is due to a finite number of sites on the enzyme,

and that when all of the sites are occupied, the maximum rate is obtained. The Michaelis-Menten expression for velocity is illustrated by the following equation:

$$v = \frac{Vmax[S]}{Km + [S]}$$

In this expression, V_m and K_m are constants that are characteristic of the enzyme. They are defined as follows:

a. The maximum velocity, $\mathbf{V_{max}}$, is the rate obtained when all of the enzyme is present as an E•S complex, with substrate bound to the active site. The V_{max} increases as the concentration of enzyme increases.

b. The $\mathbf{K_m}$ is the substrate concentration that is required to achieve half of the maximum velocity. These relationships are shown in Figure 2-9. Rough estimates of the V_{max} and K_m values can be obtained from this figure. However, values for both V_{max} and K_m obtained from this type of graph are inherently subject to large errors due to the shape of the curve; the value for V_{max} must be extrapolated from an infinitely large value for [S]. Much more accurate values of these two kinetic parameters can be obtained from secondary plots as described below. K_m is independent of the enzyme concentration.

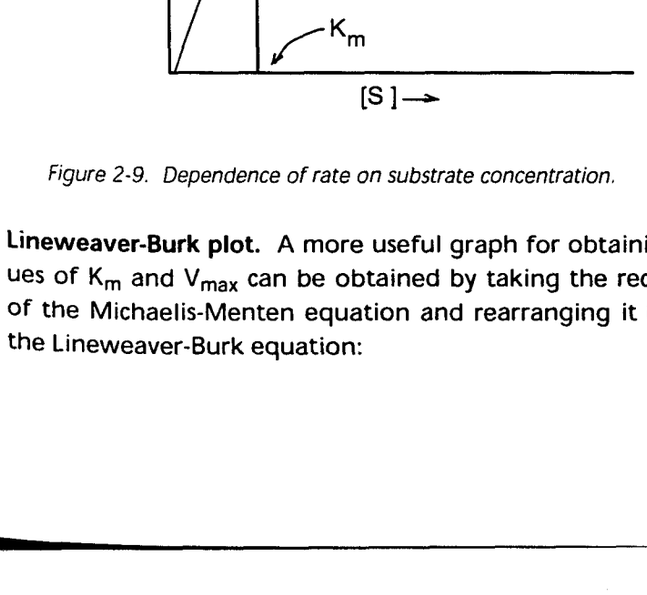

Figure 2-9. Dependence of rate on substrate concentration.

3. **Lineweaver-Burk plot.** A more useful graph for obtaining values of K_m and V_{max} can be obtained by taking the reciprocal of the Michaelis-Menten equation and rearranging it to give the Lineweaver-Burk equation:

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- ***V_{max}:*** *theoretical rate when all enzymes are working*
- ***K_m:*** *substrate concentration at which the reaction is running at half of the maximum rate (where $V = 1/2\ V_{max}$)*

NOTE

Remember that the equation for a straight line is y = mx + b, where m = slope and b = y intercept.

$$\frac{1}{V} = \frac{Km}{Vmax} \bullet \frac{1}{S} + \frac{1}{Vmax}$$

As shown in Figure 2-10, this equation describes a straight line when 1/V is plotted against 1/[S]. The slope of the line is equal to K_m/V_{max}, the intercept of the line on the y-axis is equal to $1/V_{max}$, and the intercept on the x-axis is equal to $-1/K_m$.

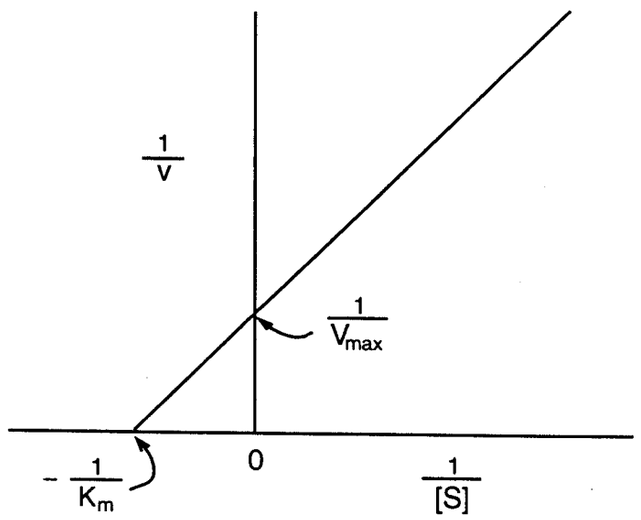

Figure 2-10. Lineweaver-Burk plot.

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Lineweaver-Burk plot

x-axis:	*1/[S]*
y-axis:	*1/V = 1/rate*
slope:	K_m/V_{max}
x-intercept:	$-1/K_m$
y-intercept:	$1/V_{max}$

NOTE

- $K_m \propto$ *1/affinity*
- $\downarrow K_m \rightarrow \uparrow$ *substrate affinity*
- $\uparrow K_m \rightarrow \downarrow$ *substrate affinity*

4. **Significance of K_m and V_{max}.** Each of these kinetic constants reflects an intrinsic property of the enzyme.
   a. **K_m** is related to the affinity of the enzyme for the substrate. The lower the value for K_m, the higher the affinity of the enzyme for the substrate, and vice versa. Frequently, the K_m value of an enzyme for its substrate is approximately equal to the substrate concentration found in the cell.
   b. **V_{max}** is related to the efficiency with which substrate is converted to product when all of the active sites are occupied. Some enzymes, such as catalase, are highly efficient, with the rate of product formation approximating the rate of substrate binding; others, like RNA polymerase, are much less efficient at converting substrate to product.

E. **Inhibitors of enzyme activity.** Much of toxicology and pharmacology is based on the principles of enzyme inhibition. Many metabolic poisons, such as insecticides and nerve gases, are enzyme inhibitors. Similarly, many drugs are inhibitors of key enzymes in metabolic pathways, and often have structures similar

to the normal enzyme substrate. There are two large classes of enzyme inhibitors: reversible and irreversible.

1. **Reversible inhibitors** alter the kinetic properties of an enzyme by binding noncovalently to the enzyme through multiple interactions with amino acid side chains. The effect of a reversible inhibitor is removed by its dissociation from the enzyme. There are three types of reversible inhibitors: competitive, noncompetitive, and uncompetitive.
   a. **Competitive inhibitors** are structural analogs of the substrate and compete with the substrate for binding to the acitve site. The effect of a competitive inhibitor can be overcome by increasing the concentration of substrate. The K_m for the substrate is increased (i.e., since both inhibitor and substrate are competing for the same site, more substrate is needed to reach half maximum velocity). V_{max} remains unchanged. Competitive inhibitors are easily identified by Lineweaver-Burk plots (Figure 2-11), in which the slope is increased but the intercept on the 1/V axis is unchanged. The intercept on the x-axis is shifted closer to zero (corresponding to an increase in K_m).

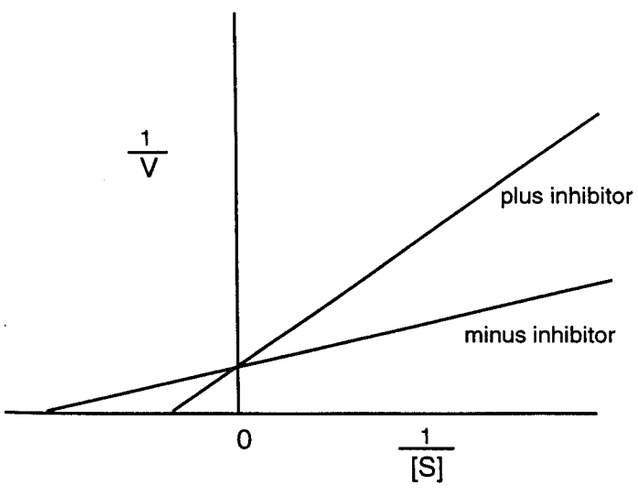

Figure 2-11. Lineweaver-Burk plot of competitive inhibition.

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Competitive inhibition:

- *$\uparrow K_m$*
- *V_{max} is unchanged*
- *Slope increases*
- *y-intercept is unchanged*
- *x-intercept shifts to the right*

   b. **Noncompetitive inhibitors** bind to some site other than the active site and are not structural analogs of the substrate. The V_{max} of the reaction is decreased, but the K_m for the substrate remains unchanged. The effect of a noncompetitive inhibitor cannot be overcome by increasing the substrate concentration. In a Lineweaver-Burk plot (Figure 2-12), the intercept on the 1/V axis is increased (correspond-

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Noncompetitive inhibition:

- *K_m is unchanged*
- *$\downarrow V_{max}$*
- *Slope increases*
- *y-intercept shifts upward*
- *x-intercept is unchanged*

ing to a decrease in V_{max}), and the intercept on the 1/[S] axis is unchanged.

c. **Uncompetitive inhibitors** bind directly to the enzyme substrate complex, but not to free enzyme. This bind causes a conformational change at the active site that renders the enzyme inactive. Both V_{max} and K_m are changed. Both V_{max} and K_m decrease, but the slope of the inhibited reaction is parallel with the slope of the uninhibited reaction (K_m/V_{max}). Lineweaver-Burk plots at various concentrations of the inhibitor will be a series of parallel lines.

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Uncompetitive inhibition:

- *K_m and V_{max} are changed*
- *Slope remains the same*
- *x-intercept shifts to the left*
- *y-intercept shifts upward*

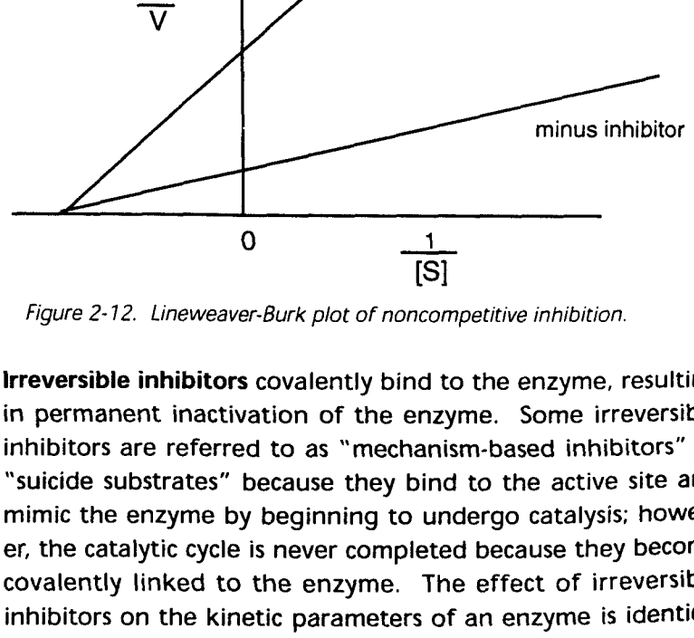


Figure 2-12. Lineweaver-Burk plot of noncompetitive inhibition.

2. **Irreversible inhibitors** covalently bind to the enzyme, resulting in permanent inactivation of the enzyme. Some irreversible inhibitors are referred to as "mechanism-based inhibitors" or "suicide substrates" because they bind to the active site and mimic the enzyme by beginning to undergo catalysis; however, the catalytic cycle is never completed because they become covalently linked to the enzyme. The effect of irreversible inhibitors on the kinetic parameters of an enzyme is identical to that of a noncompetitive inhibitor.

CLINICAL CORRELATE

Lead toxicity is caused by lead's inhibitory effect on a number of enzymes having essential sulfhydryl groups in their active sites. The sulfhydryl groups react covalently with lead, resulting in irreversible inactivation of the enzyme. Two enzymes in the pathway of heme synthesis (aminolevulinic acid dehydratase and ferrocheletase) are inactivated by low levels of lead, resulting in decreased synthesis of heme and hemoglobin and increased excretion of aminolevulinic acid. Treatment with chelators such as penicillamine decreases the toxicity by forming nontoxic complexes with lead.

F. **Regulation of enzyme activity.** Homeostasis requires that the rate of metabolic pathways be carefully regulated and coordinated with one another. This is typically achieved by the regulation of one or two key enzymes in each pathway. The key enzymes that are regulated usually catalyze either the rate-limiting reaction in the pathway or a reaction that is essentially irreversible. There are four major mechanisms for controlling the activity of enzymes.

1. **Allosteric regulation.** The activity of allosteric enzymes is regulated by the reversible binding of an effector molecule to a site other than the active site. Substrate saturation curves for allosteric enzymes are usually sigmoidal (Figure 2-13). Allosteric effectors can be either positive (activators) or negative (inhibitors) and act by altering either the K_m, V_{max}, or both. Activators either decrease the K_m or increase the V_{max}, while inhibitors increase the K_m or decrease the V_{max}. Common types of effectors include end products of pathways or molecules that reflect the energy state of the cell (ATP, ADP, AMP, NADH, NAD^+, acetyl-CoA).

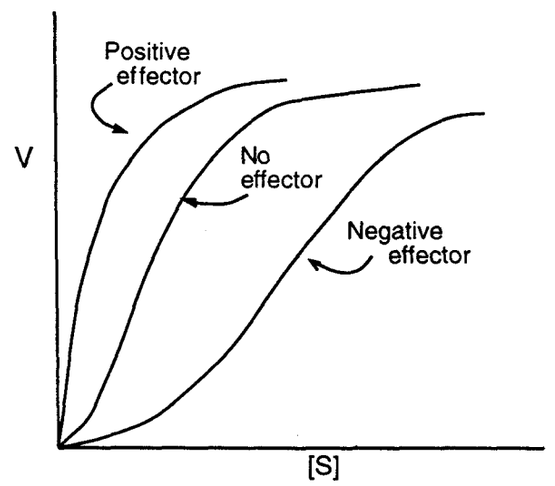


Figure 2-13. Substrate saturation curve for an allosteric enzyme.

2. **Covalent modification.** The activity of many enzymes is regulated by a phosphorylation/dephosphorylation cycle in which a specific serine, threonine, or tyrosine side chain becomes modified (Figure 2-14). The addition and removal of the phosphate group are catalyzed by a family of protein kinases and protein phosphatases that respond to hormonal stimulation of cells. Phosphorylation can increase or decrease the activity of an enzyme, or it can alter the regulatory properties.
3. **Isoenzymes.** Isoenzymes are different proteins that catalyze the same reaction, but have different catalytic and regulatory properties, and frequently differ in tissue and/or organelle specificity. The appearance of tissue-specific isoenzymes in plasma is of diagnostic value in identifying sites of tissue damage.

IN A NUTSHELL

Allosteric regulation:

- *Activators:* $\downarrow K_m$ *or* $\uparrow V_{max}$
- *Inhibitors:* $\uparrow K_m$ *or* $\downarrow V_{max}$

CLINICAL CORRELATE

Growth factors (i.e., hormones) activate receptors via tyrosine phosphorylation; the newly phosphorylated tyrosine can induce signal transduction in the cell. In many cancers, this pathway is often disregarded and the growth signal is always on, causing unregulated cell proliferation.

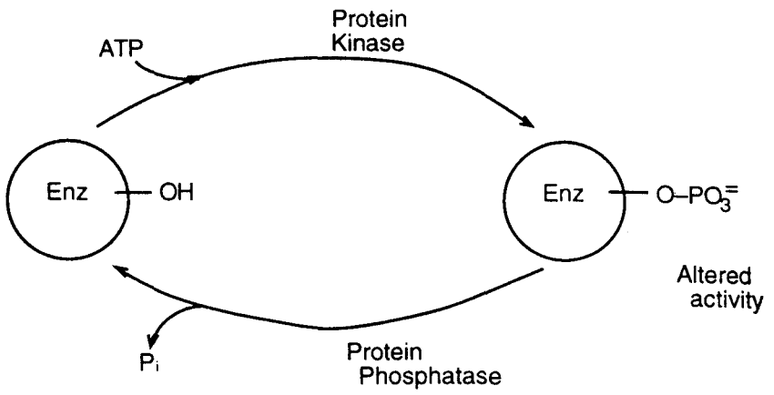


Figure 2-14. Regulation of enzyme activity by covalent modification.

4. **Induction and repression of enzyme synthesis.** Since enzyme activity is directly proportional to the amount of enzyme present, one way to regulate enzyme-catalyzed reactions is to alter the rate at which enzymes are synthesized. This type of regulation is frequently mediated by steroid or thyroid hormones that act in the nucleus to increase or decrease the rate of transcription, and secondarily, protein synthesis.

COENZYMES AND VITAMINS

Most enzymes that catalyze transfer reactions or oxidation/reduction reactions require a coenzyme that serves as an intermediate carrier of some specific functional group. Coenzymes are small organic molecules that are more stable than proteins. Coenzymes are derived from vitamins. Vitamins cannot be synthesized *de novo* by human tissues and must therefore be supplied by the diet. In addition to dietary sources, some vitamins are also synthesized by bacteria normally present in the intestinal flora. Vitamins may be classified as either water soluble or fat soluble. All of the water-soluble vitamins and some of the fat-soluble vitamins serve as precursors for coenzymes. Other fat-soluble vitamins control the rate of transcription of specific genes, thereby influencing the rate of enzyme-catalyzed reactions by altering the amount enzyme present in the cell.

A. **Water-soluble vitamins**

1. **Niacin,** also known as vitamin B_3 or nicotinic acid, is present in whole grains, meat, and nuts. Up to 50% of the body's niacin supply can be derived from the amino acid tryptophan. Niacin is converted to nicotinamide, which is then incorporated into the coenzymes **NAD+** and **NADP+**. These coenzymes are very

CLINICAL CORRELATE

Side effects of exogenous niacin use include vasodilatation, flushing, and itching. Deficiency results in pellagra, producing the clinical triad of the three D's: diarrhea, dermatitis, and dementia. (If untreated, a fourth possible "D" is death.)

important in both lipid and carbohydrate metabolism, where they act as carriers of hydride ions (two electrons and a proton) in oxidation and reduction reactions. NAD^+ is generally involved in oxidative, catabolic pathways and is more concentrated in mitochondria than in cytosol; NADPH is used in reductive, anabolic pathways and is found primarily in the cytosol.

2. **Riboflavin,** vitamin B_2, is present in organ meat, whole grains, and dairy products. The two coenzymes derived from riboflavin are **FMN** and **FAD**, both of which act as carriers of hydrogen atoms in oxidation and reduction reactions. These coenzymes are important in the oxidation of carbohydrates, lipids, and amino acids, and are found primarily in the mitochondria. Human riboflavin requirements increase with increased protein utilization during growth periods, pregnancy, lactation, and wound healing. Patients with a deficiency of riboflavin develop lesions of the lips, mouth, skin, and genitalia.
3. **Thiamine,** vitamin B_1, is present in meat, beans, peas, and grains. The coenzyme derived from thiamine is **thiamine pyrophosphate**, which functions in oxidative decarboxylation of α-ketoacids. The four major enzymes requiring thiamine pyrophosphate are pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, branch chain keto acid dehydrogenase, and transketolase.
4. **Pyridoxine,** vitamin B_6, is present in many foods, including most meats and vegetables. The coenzyme derived from this vitamin is **pyridoxal phosphate**, which acts as a carrier of amino groups in transamination, decarboxylation, racemization, and dehydration reactions. Pyridoxine deficiency may develop during pregnancy, alcoholism, or with prolonged exposure to isoniazid or penicillamide therapy, and in women on oral contraceptives. Both a deficit and excess of pyridoxine may lead to peripheral neuropathy and dermatitis.
5. **Pantothenic acid** is widely distributed in foods, particularly in meats and grains. The coenzyme derived from this vitamin is **coenzyme A**, which acts as a carrier of acyl groups and is particularly important in lipid metabolism. The key feature of this coenzyme is a sulfhydryl group that forms a high-energy thioester linkage with the carboxyl group of fatty acids. Pantothenic acid deficiency is rare.
6. **Biotin** is present in many foods, including liver, grains, and eggs. It is also synthesized by intestinal bacteria. The conversion to a coenzyme simply requires that it be covalently linked to the appropriate enzymes. Biotin acts as a carrier of "acti-

CLINICAL CORRELATE

Mild thiamine deficiency leads to peripheral neuropathy (dry beriberi). More severe vitamin depletion leads to high-output cardiac failure (wet beriberi) and the dementia, ataxia, and opthalmoplegia of the Wernicke-Korsakoff syndrome frequently seen in chronic alcoholics. (A fourth possible "D" = death.)

vated carboxyl" groups for three key enzymes that catalyze carboxylation reactions. The enzymes and the pathways they participate in are: pyruvate carboxylase (gluconeogenesis), acetyl-CoA carboxylase (fatty acid synthesis), and propionyl-CoA carboxylase (branched chain amino acid catabolism). Biotin deficiency is rare. Excessive consumption of raw egg impairs biotin absorption due to the presence of a biotin-binding protein, avidin, in egg whites. Antibiotics that alter the intestinal flora can also lead to biotin deficiency. Symptoms of deficiency include alopecia, skin and bowel inflammation, and muscle pain.

CLINICAL CORRELATE

Folic acid deficiency is most commonly seen in pregnancy and alcoholism. Deficiency states can result in megaloblastic anemia and neural tube defects. Supplementation is recommended before and during pregnancy.

7. **Folic acid** is present in liver, fresh fruit, and leafy green vegetables. The coenzyme form of this vitamin is **tetrahydrofolic acid** (TF), which acts as a carrier of one-carbon fragments in metabolism. TF carries one-carbon units (except carboxyl groups) at all stages of oxidation. The coenzyme is important in amino acid catabolism and in the synthesis of purines and pyrimidines. The donors of the one-carbon units are serine, histidine, glycine, and tryptophan; the acceptors are intermediates in the pathways of purine and pyrimidine nucleotide synthesis.

CLINICAL CORRELATE

Vitamin C deficiency results in scurvy, characterized by poor wound healing and bone formation, gum changes, and petechiae.

8. **Vitamin C,** or ascorbic acid, is found in fresh fruits and vegetables. This vitamin exists in both oxidized and reduced forms, with the reduced form being the active form. Vitamin C facilitates iron absorption from the gut and is also required in a number of hydroxylation reactions, including proline and lysine hydroxylation for collagen synthesis and dopamine hydroxylation in catecholamine synthesis. Vitamin C also serves as an antioxidant in cells.
9. **Vitamin B_{12},** or cobalamin, is synthesized exclusively by microorganisms, but is conserved in animal tissues, where it is found in high concentrations in the liver and kidney. It is required as the coenzyme for two reactions in human biochemistry: the methylation of homocysteine to methionine, and the conversion of methylmalonyl-CoA to succinyl-CoA. The absorption of vitamin B_{12} requires **intrinsic factor,** a protein synthesized by parietal cells of the gastric mucosa. A deficiency of vitamin B_{12} is rare, with the most common cause being a defect in absorption. A deficiency of vitamin B_{12} leads to megaloblastic anemia, with or without an associated nervous system neuropathy due to widespread demyelination.

B. **Fat-soluble vitamins**

1. **Vitamin A** is supplied by several sources, including yellow and orange vegetables, liver, milk, and eggs. Vitamin A exists in three forms: **retinal, retinol,** and **retinoic acid.** Retinal acts a

a cofactor for the protein opsin to form a rhodopsin complex, which acts as the light receptor in the visual process. Retinol and retinoic acid are required for growth, differentiation, and maintenance of epithelial cells; they bind to nuclear receptors and regulate the rate of transcription of specific genes. Patients with fat malabsorption or celiac disease may become vitamin A deficient, producing night blindness and dryness of the conjunctiva, cornea, and skin (hyperkeratosis). Excessive amounts of this vitamin are also toxic to humans, producing joint pain, headache, and long bone thickening.

2. **Vitamin D** is found in high concentrations in fish oils, liver, and fortified milk. It can also be synthesized in human skin by ultraviolet irradiation of sterols. The vitamin is metabolically activated by sequential hydroxylation in the liver and kidney to produce the active form of the vitamin, **1,25-$(OH)_2$-Vit D**. One of the major roles of vitamin D is to increase intestinal calcium and phosphate absorption. It binds to nuclear receptors and increases the rate of transcription of the gene coding for a protein that transports calcium from the lumen into the intestinal mucosal cell. It also acts in concert with parathyroid hormone to mobilize calcium from bone.

3. **Vitamin K** is synthesized by intestinal bacteria and is supplied by leafy green vegetables. Vitamin K acts as a coenzyme for **glutamate carboxylase,** an enzyme that catalyzes the carboxylation of glutamic acid side chains in several of the clotting factors (factors II, VII, IX, and X). A deficiency of vitamin K results in an accumulation of preprothrombin, a deficiency in prothrombin, and an increase in clotting time. Newborns are vitamin K deficient since their intestinal tract is still sterile. Antibiotic sterilization of the gut or malabsorption of fat can lead to deficiency and bleeding complications.

4. **Vitamin E,** also known as tocopherol, is found in leafy vegetables, vegetable oils, and grains. The major function of vitamin E is as an antioxidant, where its first line of defense is against peroxidation of polyunsaturated fatty acids found in cellular membranes. Fat malabsorption may lead to vitamin E deficiency. In newborns, symptoms include hemolytic anemia; in adults, sensory ataxia due to spinocerebellar degeneration may occur. In humans, vitamin E deficiency is seen frequently in premature infants and malabsorption syndromes.

CLINICAL CORRELATE

A deficiency of vitamin D occurs with lack of sunshine or renal failure, and leads to rickets in children and osteomalacia in adults. Excessive amounts of vitamin D lead to hypercalcemia and widespread calcification of soft tissues.

Bioenergetics

Cells extract energy from food by the oxidation of carbohydrates, proteins, and fats to CO_2 and H_2O. Catabolic or degradative pathways involve oxidation reactions that release energy, most of which is captured in the high-energy phosphate bonds of ATP. In contrast, anabolic (synthetic) pathways involve reduction reactions that require the input of energy, with the reducing power and energy being supplied by NADPH and ATP, respectively. The pathways for catabolism of the metabolic fuels converge at acetyl-CoA, a common intermediate in the degradation of carbohydrates, proteins, and fats. Acetyl-CoA is oxidized in the mitochondria via the citric acid cycle. The released energy is conserved as electron pairs that are transferred from acetyl-CoA to NADH and $FADH_2$. The electrons are then transferred sequentially from NADH and $FADH_2$ to O_2 , resulting in the formation of H_2O. The oxidation of NADH and $FADH_2$ by molecular oxygen, a process catalyzed by the electron transport chain, is a highly exergonic process. The energy released is used to drive the phosphorylation of ADP to form ATP, an endergonic reaction. Most of the ATP supply in the cell is derived from oxidative phosphorylation. The regulation of oxidative phosphorylation and the citric acid cycle are closely linked—they are both dependent upon the availability of molecular oxygen. This chapter will review the principles of thermodynamics and oxidation-reduction reactions that form the basis for energy metabolism.

METABOLIC SOURCES OF ENERGY

Energy is extracted from food via oxidation, resulting in the end products of carbon dioxide and water. This process occurs in four

stages (Figure 3-1). In the first stage, metabolic fuels are hydrolyzed to a diverse set of monomeric building blocks, including glucose, amino acids, and fatty acids. In the second stage, the building blocks are degraded by various pathways to a common metabolic intermediate, **acetyl-CoA.** Most of the energy contained in metabolic fuels is conserved in the chemical bonds (electrons) of acetyl-CoA. In stage three, **the citric acid (Krebs, or tricarboxylic acid, TCA) cycle** oxidizes acetyl-CoA to CO_2, and the electrons pairs present in the carbon-carbon and carbon-hydrogen bonds are transferred to the electron carriers, NADH and $FADH_2$. The final stage in the extraction of energy from food is **oxidative phosphorylation,** where the energy in the electron pairs of NADH and $FADH_2$ is released via the **electron transport chain** (ETC) and is used to synthesize ATP.

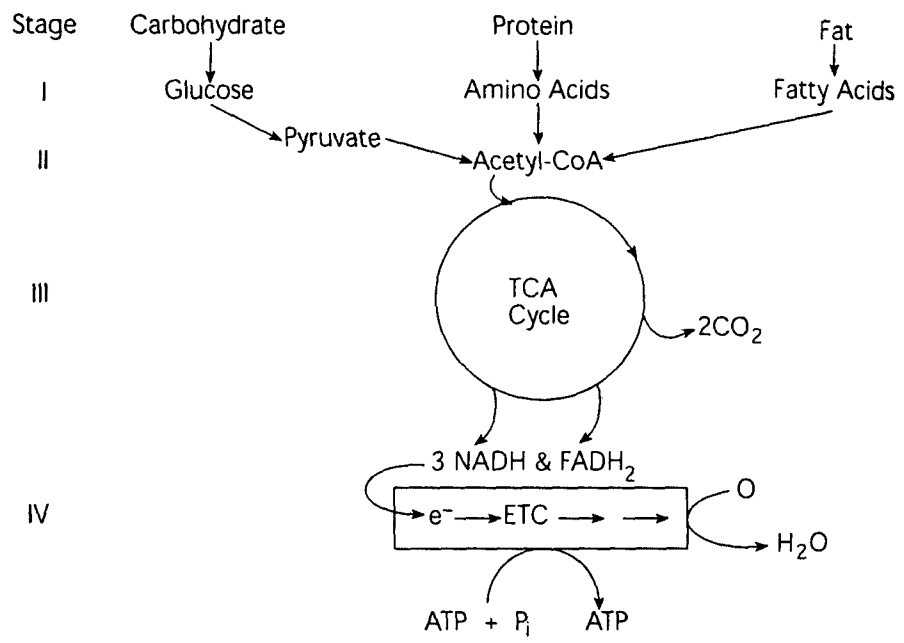


Figure 3-1. Extraction of energy from metabolic fuels.

THERMODYNAMIC PRINCIPLES

The principles underlying energy metabolism are based on the thermodynamics of chemical reactions. Some of the variables involved are:

G = free energy (energy available to do work)
H = enthalpy (heat content of a compound)
S = entropy (randomness of a system)
T = absolute temperature (measured in °K)
R = gas constant (1.987 cal/mol•degree)
F = Faraday's constant (23 kcal/volt•mol)

A. **Free energy change (ΔG) of reactions.** The free energy change of a system is the portion of the total energy that is available for useful work. For any chemical reaction, the ΔG is equal to the difference in energy between the products and the reactants. The free energy change predicts the direction in which a reaction will proceed spontaneously.

$$\Delta G = \Delta H - T\Delta S = G_{products} - G_{reactants}$$

The standard free energy change (ΔG°) is a constant for any given reaction. (The superscript indicates "standard conditions," which are pH 7, 25°C, and all reactants and products at 1.0M concentration.) The ΔG° is related to the equilibrium constant, K_{eq}. For the reaction

$$A + B \longrightarrow C + D$$

the equilibrium constant is defined as:

$$K_{eq} = \frac{[C]\,[D]}{[A]\,[B]}$$

$$\Delta G^{\circ} = -RT \ln K_{eq} = -2.3\ RT \log K_{eq}$$

Therefore, if the concentration of reactants and products at equilibrium are known, the K_{eq} and the ΔG° for the reaction can be calculated.

B. **Exergonic and endergonic reactions** are distinguished on the basis of whether the ΔG of the reaction is positive or negative. Exergonic reactions have a negative ΔG, and will proceed spontaneously in the direction written. In contrast, endergonic reactions have a positive ΔG and require the input of energy to proceed in the direction written. If ΔG = 0, the reaction is at equilibrium, and the rates of the forward and the reverse reactions are equal.

C. **Coupled reaction systems.** Endergonic reactions in metabolism frequently proceed by being coupled to an exergonic reaction. The requirement for a coupled reaction system is that the product of the first reaction must be the substrate for the second reaction. Many enzyme-catalyzed reactions that use ATP are examples of coupled reactions, where the energy released by the hydrolysis of ATP is used to drive an endergonic reaction. For example, con-

IN A NUTSHELL

- *$\Delta G < 0 \rightarrow$ spontaneous reaction*
- *$\Delta G > 0 \rightarrow$ non-spontaneous reaction*
- *$\Delta G = 0 \rightarrow$ equilibrium*

sider the phosphorylation of glucose to glucose-6-phosphate, a reaction that occurs in all cells and is catalyzed by hexokinase.

$$\text{Glucose} + P_i \longrightarrow \text{Glucose-6-phosphate} \qquad \Delta G' = +3.3 \text{ kcal/mol}$$

Since this reaction is endergonic, it will not proceed spontaneously in the direction of glucose-6-phosphate formation unless energy is supplied. However, if the formation of glucose-6-phosphate is coupled to the hydrolysis of ATP, the sum of the two reactions is exergonic.

NOTE

Cells often couple energetically unfavorable reactions with ATP hydrolysis to force the reaction to proceed.

	$ATP + H_2O \longrightarrow ADP + P_i$		$\Delta G' = -7.3$ kcal/mol
	$\text{Glucose} + P_i \longrightarrow \text{Glucose-6-phosphate}$		$\Delta G' = +3.3$ kcal/mol
Sum:	$\text{Glucose} + ATP \longrightarrow \text{Glucose-6-phosphate} + ADP$		$\Delta G' = -4.0$ kcal/mol

The function of hexokinase, therefore, is to couple these two reactions so that the formation of glucose-6-phosphate is thermodynamically favorable.

D. **Central role of adenine nucleotides in energy transduction.** Some of the common phosphate-containing compounds found in cells and the energy released by hydrolysis of their phosphate bonds under standard conditions are shown in Table 3-1.

Compound	$\Delta G'$ (kcal/mol)
Phosphoenolpyruvate	−14.8
1,3-Bisphosphoglycerate	−11.8
Creatine phosphate	−10.3
Pyrophosphate	−8.0
ATP	−7.3 ←
Glucose-1-phosphate	−5.0
Fructose-6-phosphate	−3.8
AMP	−3.4
Glucose-6-phosphate	−3.3
Glycerol-3-phosphate	−2.2

Table 3-1. Energy of hydrolysis of phosphate compounds.

The positioning of these compounds in the table illustrates important concepts in energy transfer reactions. Note the structure of ATP:

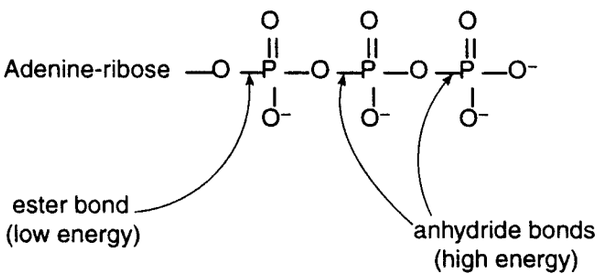


Figure 3-2. Structure of ATP.

The energy in the terminal anhydride bond of ATP is greater than the energy found in the compounds listed below it in the table, and, therefore, can be used to drive the synthesis of these compounds. Conversely, the compounds listed above ATP have more energy in their phosphate bonds than does ATP, thus allowing these compounds to transfer their phosphate groups to ADP with the formation of ATP. The conversion of ADP to ATP by the use of high-energy phosphate metabolites is known as **substrate-level phosphorylation.** As shown in Table 3-1, there are three phosphorylated intermediates in cells with sufficient energy to participate in substrate-level phosphorylation. Phosphoenol-pyruvate and 1,3-bisphosphoglycerate are intermediates in glycolysis, and creatine phosphate serves as a reservoir of high-energy phosphate bonds in muscle.

E. **Other high-energy carriers.** Many chemical groups that are transferred in metabolic reactions require "high-energy carriers" for the reaction to be exergonic. Examples of some groups that are transferred, their high-energy carriers, and the types of reactions and/or pathways in which they participate are summarized in Table 3-2.

Group	Carrier	Pathways/reactions
P_i	ATP	Kinase reactions
Sugars	UDP-sugar	Polysaccharide synthesis
Acetate	Acetyl-CoA	Fatty acid synthesis
Fatty acids	Acyl-CoA	Triacylglycerol synthesis
Amino acids	AMP-amino acid	Protein synthesis
Methyl	SAM[a]	Methylation reactions
Carboxyl	Carboxy-biotin	Carboxylation reactions
Sulfate	PAPS[b]	Sulfation reactions

[a]S-adenosylmethionine; [b]Phosphoadenosine phosphosulfate

Table 3-2. High-energy carriers of chemical groups in metabolism.

IN A NUTSHELL

ATP formation via substrate-level phosphorylation occurs with:

- *Phosphoenolpyruvate* } *glycolysis*
- *1,3-Bisphosphoglycerate* } *glycolysis*
- *Creatine phosphate → muscle*

OXIDATION-REDUCTION REACTIONS

The principles of oxidation and reduction are an integral part of energy conservation in cells. The energy contained in metabolic fuels is released through a series of oxidation-reduction (redox) reactions that occur mainly in the mitochondria. Approximately forty percent of this energy is conserved as ATP.

MNEMONIC

LEO** the lion says **GER:
***l**oss of **e**lectrons = **o**xidation*
***g**ain of **e**lectrons = **r**eduction*
OIL RIG:
***o**xidation **i**s **l**oss (of electrons)*
***r**eduction **i**s **g**ain (of electrons)*

A. **General principles.** Oxidation-reduction reactions involve the transfer of electrons between a donor and an acceptor.

1. **Definitions. Oxidation** is defined as the loss of electrons, while **reduction** is defined as the gain of electrons. Every oxidation is accompanied by a reduction, each of which is considered to be a half-reaction.
2. The **standard reduction potential, E°,** is a constant that describes the tendency of a compound to be reduced. It is expressed in volts and is measured under standard conditions defined as 25° C, pH 7, and at concentrations of 1.0M for electron donors and acceptors. Values of standard reduction potentials for some common half-reactions are shown in Table 3-3. Stronger electron donors have a more negative reduction potential. Thus, under standard condtions, NADH with an E° of –0.32 volt will reduce pyruvate (or any other compound with a less negative E°).

Half-reaction			E°(V)
$NAD^+ + 2e^- + H^+$	→	NADH	–0.32
Pyruvate + $2e^- + 2H^+$	→	Lactate	–0.19
Oxaloacetate + $2e^- + 2H^+$	→	Malate	–0.17
FAD + $2e^- + 2H^+$	→	$FADH_2$	–0.06
CoQ + $2e^- + 2H^+$	→	$CoQH_2$	+0.10
Fumarate + $2e^- + 2H^+$	→	Succinate	+0.13
Cyt a (Fe^{3+}) + e^-	→	Cyt a (Fe^{2+})	+0.29
1/2 O_2 + $2e^- + 2H^+$	→	H_2O	+0.82

Table 3-3. Standard reduction potentials for common redox pairs. Note that all of the reactions are written as reductions.

3. **Relationship between ΔE° and ΔG°.** The change in the standard reduction potential for an oxidation-reduction reaction is related to the standard free energy change (ΔG°) for the reaction by the expression shown below, where n is the number of electrons transferred, F is the Faraday constant, and $\Delta E^{\circ} = E^{\circ}_{\text{electron acceptor}} - E^{\circ}_{\text{electron donor}}$.

$$\Delta G^{\circ} = -nF\Delta E^{\circ}$$

NOTE

If the reduction potential is given, reverse the sign to find the oxidation potential: $E^{\circ}_{red} = -0.32 \rightarrow E^{\circ}_{ox} = +0.32$

From this relationship, it is clear that for an oxidation-reduction reaction to be exergonic and to proceed spontaneously, ΔE° must be a positive value.

4. **Example.** These thermodynamic principles can be illustrated by considering the transfer of electrons from NADH to molecular oxygen. In this example, NADH is the electron donor (it has the more negative standard reduction potential) and oxygen is the electron acceptor. The overall oxidation-reduction reaction can be written as the sum of two half reactions.

Oxidation of NADH:	$NADH \longrightarrow NAD^+ + H^+ + 2e^-$	$\Delta E^{\circ} = +0.32$ volt
Reduction of O:	$1/2\ O_2 + 2\ H^+ + 2e^- \longrightarrow H_2O$	$\Delta E^{\circ} = +0.82$ volt
Overall reaction:	$NADH + 1/2\ O_2 + H^+ \longrightarrow NAD^+ + H_2O$	$\Delta E^{\circ} = +1.14$ volt

The positive value of ΔE° indicates that the reaction will proceed spontaneously. As defined above, the change in standard free energy can be calculated (n = 2 electrons being transferred, and F = 23 kcal/volt)

$$\Delta G^{\circ} = -nF\Delta E^{\circ}$$

$$\Delta G^{\circ} = -(2)(23\ \text{kcal/volt})(1.14\ \text{volt}) = -52.4\ \text{kcal}$$

Since ΔG° is negative, the oxidation of NADH by oxygen is an exergonic reaction. This exergonic reaction is the overall reaction catalyzed by the electron transport chain. The electrons in NADH are transferred sequentially through a series of carriers to oxygen, where each electron carrier is reduced by the preceding carrier.

3. **Major electron carriers.** A wide variety of dehydrogenases participate in the oxidation of metabolic fuels. Most of these enzymes use either NAD^+ or FAD as electron acceptors. The major carrier of electrons in reductive biosynthetic reactions is NADPH.

1. **NAD^+** is the electron accceptor in reactions involving oxidation of hydroxylated carbon atoms (Figure 3-3). NAD^+ accepts a hydride ion, H^- (two electrons and a proton) to form NADH, and the hydroxyl group is oxidized to a carbonyl group.

Figure 3-3. The reduction of NAD^+ to NADH.

IN A NUTSHELL

- *Electron acceptors* → *NAD^+, FAD*
- *Electron donors* → *NADPH, NADH*

2. **FAD** is the electron acceptor in reactions involving the oxidation of two adjacent carbons, resulting in the formation of a carbon-carbon double bond (Figure 3-4). A hydrogen atom is removed from each carbon atom and is transferred to FAD to form $FADH_2$.

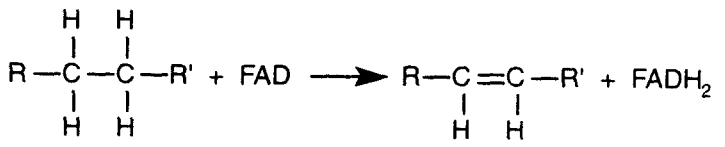


Figure 3-4. The reduction of FAD to $FADH_2$.

3. **NADPH** is the major source of reducing power for biosynthetic pathways. In contrast to NADH, which is generated and used primarily in the mitochondria, most of the NADPH is formed and used in extramitochondrial reactions (described in Chapter 4).

CITRIC ACID CYCLE

The **citric acid cycle,** also known as the **tricarboxylic acid (TCA) cycle** or the **Krebs cycle,** is localized in the mitochondria.

A. **Functions.** The pathway is amphibolic, playing important roles in both catabolic and anabolic pathways.

1. **Catabolic function.** The major catabolic function of the citric acid cycle is to transfer electron pairs (potential energy) from acetyl-CoA to NADH and $FADH_2$. With every turn of the cycle, two carbon atoms enter as acetyl-CoA, two carbon atoms leave as CO_2, and the four pairs of electrons in the carbon-hydrogen and the carbon-carbon bonds are transferred to NADH and $FADH_2$. The overall reaction for the oxidation of acetyl-CoA is shown in Figure 3-5.

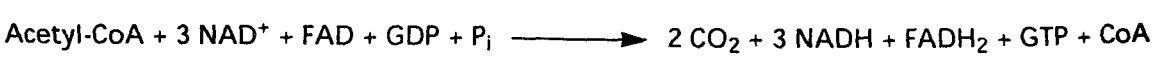


Figure 3-5. Stoichiometry of the citric acid cycle.

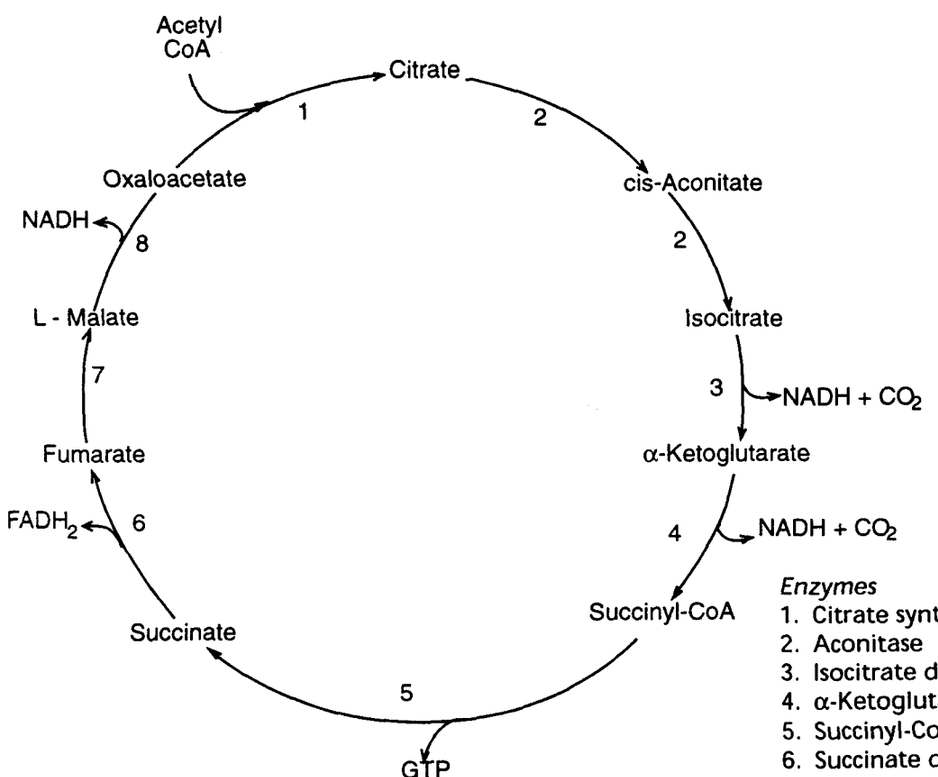
One high-energy phosphate bond (GTP) is produced by substrate level phosphorylation. Assuming adequate oxygen is available, subsequent oxidation of NADH and $FADH_2$ will result in the synthesis of 11 molecules of ATP by oxidative phosphorylation (described below). Therefore, the complete oxidation of one molecule of acetyl-CoA results in the synthesis of 12 ATP equivalents.

2. **Anabolic functions.** Intermediates in the citric acid cycle are used as substrates for various biosynthetic pathways.
   a. Citrate is a substrate for fatty acid synthesis.
   b. Oxaloacetate is the first intermediate in gluconeogenesis.
   c. Succinyl-CoA is required for the synthesis of heme.
   d. Oxaloacetate and α-ketoglutarate are substrates for amino acid synthesis

B. **Reactions of the citric acid cycle.** The reactions by which acetyl-CoA is oxidized to CO_2 are shown in Figure 3-6.

NOTE

Some amino acids can be converted to α-ketoglutarate and can enter the citric acid cycle at this intermediate.



Enzymes
1. Citrate synthase
2. Aconitase
3. Isocitrate dehydrogenase
4. α-Ketoglutarate dehydrogenase
5. Succinyl-CoA thiokinase
6. Succinate dehydrogenase
7. Fumarase
8. Malate dehydrogenase

Figure 3-6. The citric acid cycle.

The entry of acetyl-CoA into the cycle involves condensation with oxaloacetate to produce **citrate**, a C_6 tricarboxylic acid. Following isomerization to **isocitrate**, two sequential oxidative decarboxylation reactions occur, resulting in the formation of **succinyl-CoA**. Each of these reactions releases CO_2 and transfers electrons to NADH. Succinyl-CoA contains a thioester linkage that has suffi-

MNEMONIC

***C**itric **a**cid **i**s **K**reb's **s**tarting **s**ubstrate **f**or **m**itochondrial **o**xidation:*

*citrate, cis-**a**conitate, **i**socitrate, α-**k**etoglutarate, **s**uccinyl-CoA, **s**uccinate, **f**umarate, **m**alate, **o**xaloacetate*

IN A NUTSHELL

Enzyme	***Inhibitors***	***Activators***
Pyruvate dehydrogenase	*ATP* *Acetyl-CoA* *NADH*	*ADP* *CoASH* *NAD^+* *Ca^{2+}* *Insulin*
Citrate synthase	*ATP*	
Isocitrate dehydrogenase	*ATP* *NADH*	*ADP*
α-Ketoglutarate dehydrogenase	*Succinyl-CoA* *NADH* *ATP*	

cient energy to drive the synthesis of a high-energy phosphate bond. Thus in the next reaction, hydrolysis of succinyl-CoA is coupled with the synthesis of GTP from GDP and P_i. GTP is energetically equivalent to ATP and the two molecules are interconvertible. The remaining reactions complete the cycle by regenerating **oxaloacetate.** They include two oxidation reactions: the oxidation of **succinate** to **fumarate,** producing $FADH_2$; and the oxidation of **malate** to oxaloacetate, producing NADH. All of the enzymes of the cycle are located in the mitochondrial matrix except for succinate dehydrogenase, which is embedded in the inner mitochondrial membrane. The localization of these enzymes provides for efficient transfer of electrons from the carriers, NADH and $FADH_2$, to the electron transport chain.

C. **Key enzymes.** The four dehydrogenases (isocitrate, α-ketoglutarate, succinate, and malate) all catalyze oxidation reactions. In the isocitrate and α-ketoglutarate dehydrogenase reactions, decarboxylation also occurs. The rate at which the cycle operates is dependent on **isocitrate dehydrogenase**, the enzyme that catalyzes the rate-limiting step in the cycle. This enzyme is allosterically inhibited by ATP and NADH and is activated by ADP. Secondary sites of regulation are α-ketoglutarate dehydrogenase and citrate synthase, which are also inhibited by ATP and NADH. These three enzymes catalyze reactions that are essentially irreversible.

D. **Anaplerotic ("filling-up") reactions**. When intermediates of the cycle are removed for synthetic purposes, they must be replenished in order to ensure that acetyl-CoA can continue to be oxidized. The most important reaction for replenishing the cycle with intermediates is the conversion of pyruvate to oxaloacetate. This reaction, catalyzed by pyruvate carboxylase, requires biotin and bicarbonate as substrates, and it has an absolute requirement for acetyl-CoA as an allosteric activator (Figure 3-7). The accumulation of acetyl-CoA, due to insufficient levels of oxaloacetate, activates the synthesis of this intermediate.

$$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightarrow[\text{pyruvate carboxylase}]{\text{biotin}} \text{Oxaloacetate} + \text{ADP} + P_i$$

$$\oplus\text{acetyl-CoA}$$

Figure 3-7. The pyruvate carboxylase reaction.

ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

The final step in the aerobic oxidation of metabolic fuels is achieved by the electron transport chain (ETC). The ETC accepts electrons from

NADH and $FADH_2$ and passes them, through a series of carriers, to molecular oxygen, forming water. The energy released at specific steps in the ETC is used to synthesize ATP via a process called **oxidative phosphorylation**. All of the enzymes and cofactors required for electron transport and oxidative phosphorylation are localized in the inner mitochondrial membrane.

A. **The electron transport chain**

1. **Components.** The ETC is composed of a specific sequence of enzymes and their coenzymes, including NAD^+ and FAD-linked dehydrogenases, iron-sulfur proteins (FeS), coenzyme Q, and several cytochromes (Figure 3-8). The components of the chain can be separated into four protein-lipid complexes (I, II, III, and IV) and two mobile components (CoQ and cyt c) that move freely in the lipid bilayer.

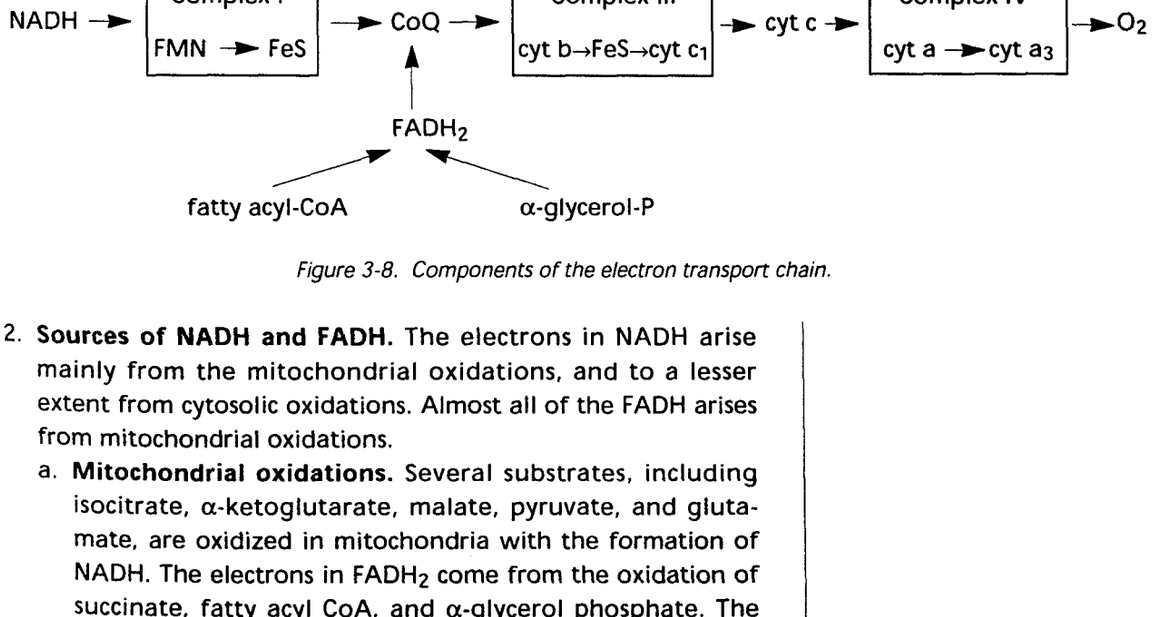


Figure 3-8. Components of the electron transport chain.

2. **Sources of NADH and FADH.** The electrons in NADH arise mainly from the mitochondrial oxidations, and to a lesser extent from cytosolic oxidations. Almost all of the FADH arises from mitochondrial oxidations.
   a. **Mitochondrial oxidations.** Several substrates, including isocitrate, α-ketoglutarate, malate, pyruvate, and glutamate, are oxidized in mitochondria with the formation of NADH. The electrons in $FADH_2$ come from the oxidation of succinate, fatty acyl CoA, and α-glycerol phosphate. The electrons from NADH and $FADH_2$ enter at different positions along the electron transport chain. Electrons from NADH enter at complex I, whereas electrons from several FAD-linked dehydrogenases enter at CoQ. Note that com-

plex II is succinate dehydrogenase, the same enzyme utilized in the citric acid cycle.

b. **Shuttles for getting cytoplasmic NADH electrons into mitochondria.** Although most oxidations occur in the mitochondria, there are a few oxidative reactions in the cytosol that produce NADH. The mitochondrial membrane, however, is impermeable to NADH; thus, the transfer of electrons from cytosolic NADH into the mitochondria for oxidation by the electron transport chain requires special shuttle systems. Two types of shuttles are found in cells, the **malate shuttle** and the **α-glycerol phosphate shuttle** (Figure 3-9). In these shuttles, malate and α-glycerol phosphate act as carriers of electrons across the inner mitochondrial membrane. In the malate shuttle, electrons originating in the cytosol are incorporated into mitochondrial NADH, whereas in the α-glycerol phosphate shuttle, cytosolic electrons are incorporated into mitochondrial $FADH_2$.

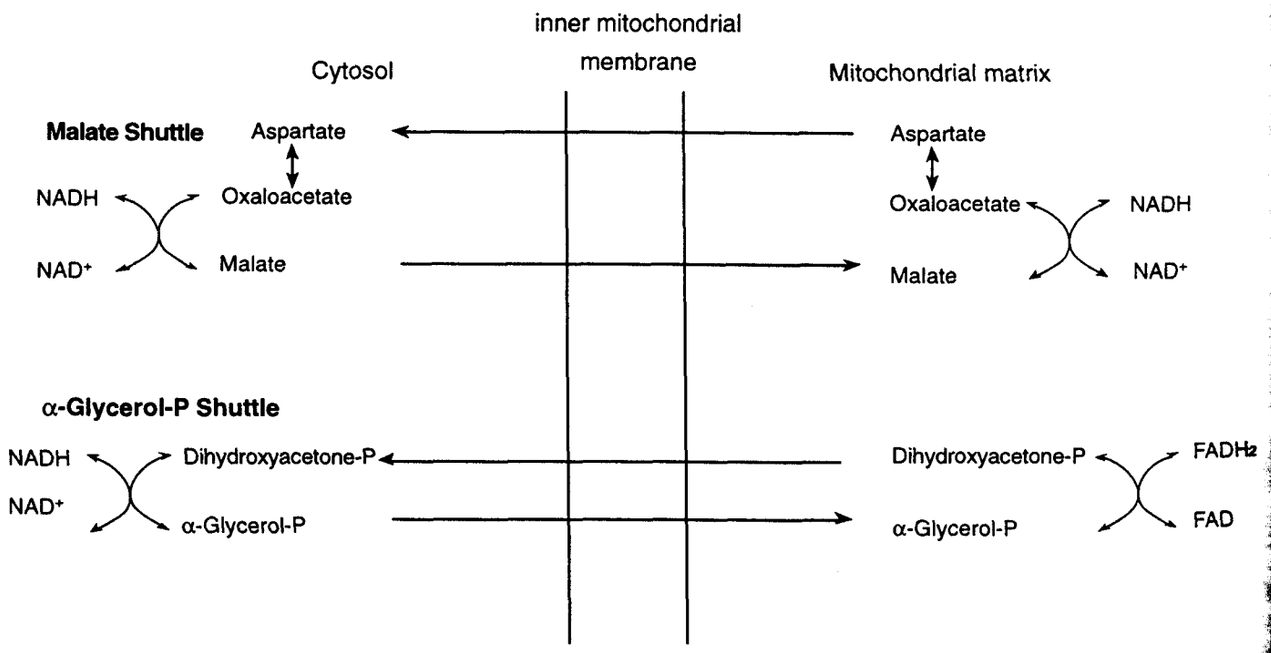


Figure 3-9. Shuttles for getting cytosolic NADH electrons into the mitochondria.

3. **Energetics.** A simplified version of the electron transport chain is shown in Figure 3-10. The carriers in the electron transport chain are arranged so that the reduction potential progressively increases from negative to positive values. Thus, as electrons move through each complex to the mobile components

of the chain, the $\Delta E' > 0$ and the $\Delta G' < 0$, ensuring that electrons flow spontaneously to oxygen.

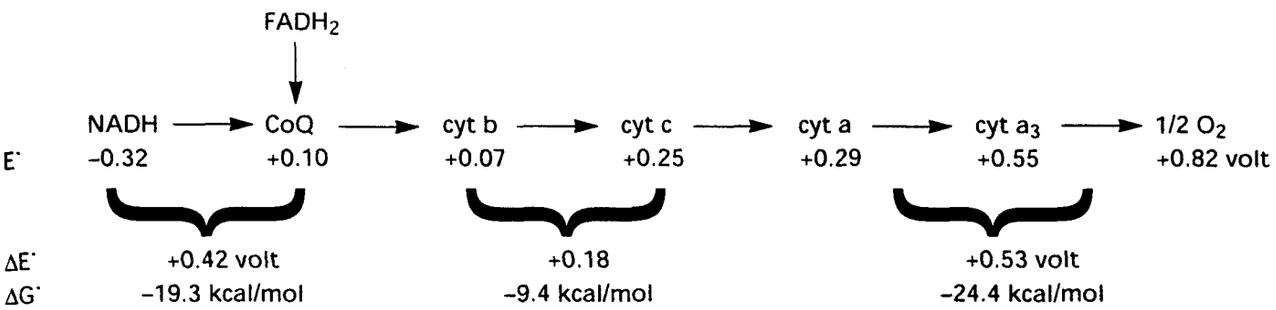


Figure 3-10. Energy released by electron flow through the ETC.

B. **Oxidative phosphorylation.** The energy required for synthesis of ATP is 7.3 kcal/mol. It is clear from Figure 3-10 that transfer of electrons from 1 mol of NADH through complexes I, III, and IV releases sufficient energy to drive the synthesis of ATP. Thus, it is at these three sites in the electron transport chain that energy can be harnessed for oxidative phosphorylation. Note that electrons from $FADH_2$ bypass complex I and flow through only two of these energy transduction sites.

1. **P/O ratio.** By definition, the P/O ratio is the number of ATP molecules produced per O atom reduced. Thus, substrates that are oxidized with the generation of NADH (malate, isocitrate, α-ketoglutarate) support the synthesis of 3 molecules of ATP and have P/O ratios of 3. Substrates that are oxidized with the production of $FADH_2$ (succinate, α-glycerol phosphate) have P/O ratios of 2.

2. **Efficiency.** The calculations shown below indicate that the oxidation of NADH by the ETC releases a total of 53 kcal of energy.

$$\Delta E' = E'_{\text{oxygen}} - E'_{\text{NADH}} = +0.82 - (-0.32) = 1.14 \text{ volts}$$
$$\Delta G' = -nF\Delta E' = -(2)(23 \text{ kcal/volt})(1.14 \text{ volt}) = -53 \text{ kcal}$$

The synthesis of ATP requires 7.3 kcal. Thus, in theory, the oxidation of one mole of NADH should release sufficient energy to drive the synthesis of 7 moles of ATP. However, since only three moles of ATP are synthesized per mole of NADH oxidized, the efficiency of oxidative phosphorylation is approximately only 40%. The remainder of the energy is released as heat.

3. **Mechanism of energy transduction.** The chemiosmotic hypothesis is the most widely accepted theory for how electron transport is coupled with ATP synthesis. This theory is based on the observation that when electrons are flowing through the ETC,

IN A NUTSHELL

	P/O ratio
NADH	*3*
$FADH_2$	*2*

DNP (2,4-dinitrophenol, an uncoupler that dissipates the H^+ gradient) has a P/O ratio of 0.

complexes I, III, and IV are pumping protons out of the matrix, creating a proton gradient across the inner mitochondrial membrane. Complex II does not pump protons. The **chemiosmotic hypothesis** asserts that the protomotive force associated with the proton gradient drives the synthesis of ATP. The movement of protons down the gradient as they reenter the matrix releases energy that is available for ATP synthesis. **ATP synthase,** also known as **complex V,** is associated with the inner mitochondrial membrane in close proximity to the electron transport chain (Figure 3-11).

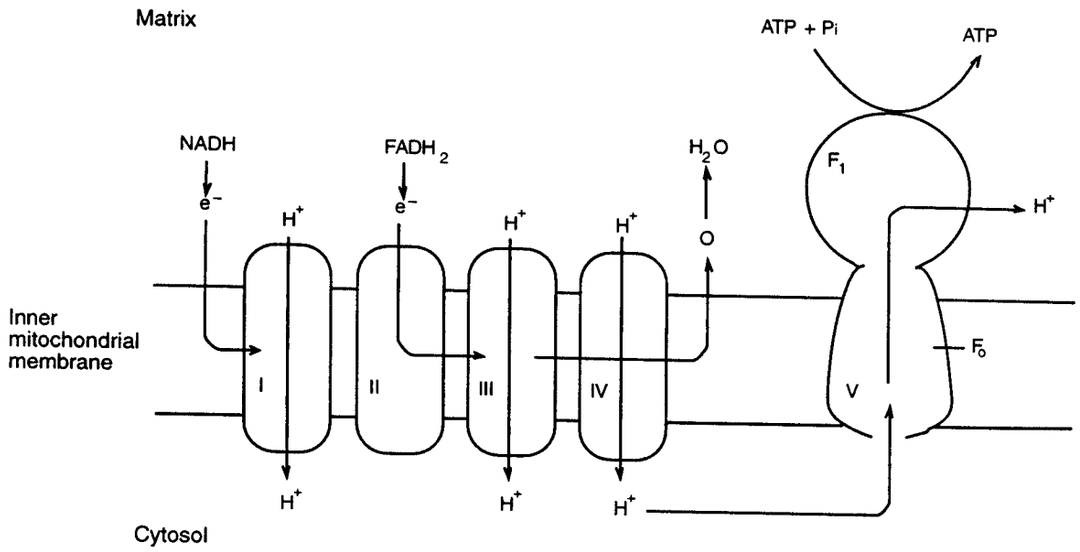


Figure 3-11. Structure of mitochondrial ATP synthase.

ATP synthase consists of two types of subunits. The F_0 subunits span the membrane, creating a proton channel that allows protons to move back into the matrix. The F_1 subunit protrudes into the matrix and, in the presence of a proton gradient, catalyzes the condensation of ADP and P_i to form ATP.

COORDINATE REGULATION OF THE CITRIC ACID CYCLE AND OXIDATIVE PHOSPHORYLATION

The rates of oxidative phosphorylation and the citric acid cycle are closely coordinated, and are dependent mainly upon the availability of O_2 and ADP. If O_2 is limited, the rate of oxidative phosphorylation decreases, and the concentrations of NADH and $FADH_2$ increase. The accumulation of NADH, in turn, inhibits the citric acid cycle. The coordinated regulation of these pathways is known as

respiratory control." In the presence of adequate O_2, the rate of oxidative-phosphorylation is dependent upon the availability of ADP. The concentrations of ADP and ATP are reciprocally related; an accumulation of ADP is accompanied by a decrease in ATP and the amount of energy available to the cell. Therefore, ADP accumulation signals the need for ATP synthesis. ADP allosterically activates isocitrate dehydrogenase, thereby increasing the rate of the citric acid cycle and the production of NADH and $FADH_2$. The elevated levels of these reduced coenzymes, in turn, increases the rate of electron transport and ATP synthesis.

LAN

Carbohydrates

Carbohydrates participate in many metabolic pathways and serve as structural components of cells and tissues. In human biochemistry, there are three major classes of carbohydrates: monosaccharides, disaccharides, and polysaccharides. The most important monosaccharide in human metabolism is glucose. All cells and tissues require the use of some glucose for energy. The brain relies almost entirely on glucose for its energy, and red blood cells derive all of their energy from glucose. Following a high-carbohydrate meal, glycogenesis in liver and skeletal muscle provides a pathway for storage of excess glucose. Conversely, the mobilization of glucose from glycogen is achieved via glycogenolysis. Gluconeogenesis is the pathway for *de novo* synthesis of glucose from amino acids, glycerol, propionate, and lactate. This chapter will emphasize the role that these pathways play in homeostasis, the tissues where they occur, the key enzymes involved, and the mechanisms for regulation. Numerous clinical examples will be provided that stress the importance of carbohydrates in both health and disease.

CLASSIFICATION

Carbohydrates are chains of carbon atoms with attached hydrogen and hydroxyl groups. The length of the carbon chain may vary, and carbohydrates can generally be represented by the formula $C_n(H_2O)_n$, where "n" is a minimum of three carbons. This formula emphasizes the idea that this class of molecules are "hydrates of carbon." In some specialized monosaccharides, one of the hydroxyl groups may be replaced by another chemical group, such as a hydrogen atom (deoxyribose in DNA), an amino group (glucosamine in

proteoglycans), or phosphate groups (most intermediates of carbohydrate metabolism pathways).

A. **Monosaccharides.** The simplest carbohydrates are the monosaccharides. Most monosaccharides in human metabolism are trioses (C_3), pentoses (C_5), and hexoses (C_6). Monosaccharides containing an aldehyde are known as **aldoses**, while those containing a keto group are called **ketoses**. The most important monosaccharide is **D-glucose,** a hexose and an aldose whose structure is shown in Figure 4-1.

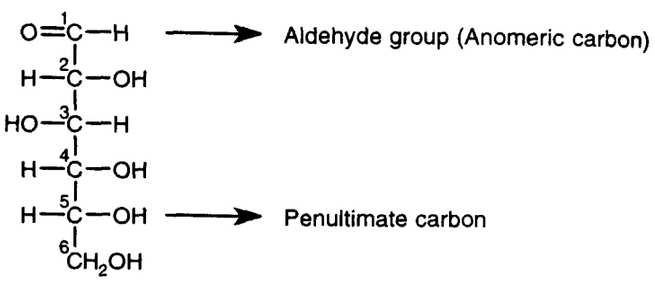


Figure 4-1. Structure of D-glucose.

1. **Nomenclature and definitions**. The numbering system for monosaccharides always starts with the carbon atom nearest the carbonyl group ($\geq C=O$).
   a. **Anomeric carbon.** The carbonyl carbon is defined as the anomeric carbon. This carbon participates in internal ring structures and glycosidic bonds between monosaccharides (discussed below).
   b. **Penultimate carbon.** The penultimate carbon is the next-to-the-last carbon in the chain. For hexoses, it is C-5 and for pentoses it is C-4.
   c. **D and L isomers.** The "D" designation describes the configuration around the penultimate carbon. If the hydroxyl group is on the right, it is a D-sugar; if it is on the left, it is an L-sugar. Almost all important monosaccharides in human biochemistry have the D-configuration.
   d. **Epimers.** Two monosaccharides are epimers if they differ in the configuration around a single carbon atom. For example, galactose is a 4-epimer of glucose. Thus, knowing the structure of glucose allows you to automatically recognize the structure of galactose. Enzymes that catalyze the interconversions of these compounds are epimerases.
   e. **Aldose-ketose isomers.** Glucose, an aldose, and fructose a ketose, differ only in the position of the carbonyl group. In glucose, the carbonyl group is at C-1, whereas in fructose it is at C-2.

2. **Cyclic structure of monosaccharides.** The straight chain structure of monosaccharides exists in equilibrium with a ring structure, resulting from the reaction of the hydroxyl of the penultimate carbon with the anomeric carbon. The cyclic structure of glucose is a six-member ring (a pyranose) that can be drawn in one of two ways (Figure 4-2).

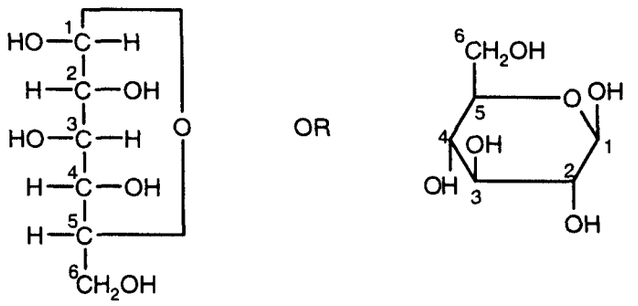


Figure 4-2. Cyclic structures for D-glucose.

In the structure on the right, the ring projects from the plane of the paper and the hydroxyl and hydrogen groups project up and down from the carbons in the ring. By convention, hydroxyl groups on the right point down and those on the left point up. When a ring structure is formed, the anomeric carbon can exist in two configurations, α and β. In α-D-glucose, the hydroxyl group on C-1 points down, whereas in β-D-glucose it points up. The predominant form of glucose in the body is β-D-glucose. D-fructose, shown in Figure 4-3, is an important hexose that forms a five-member ring (a furanose).

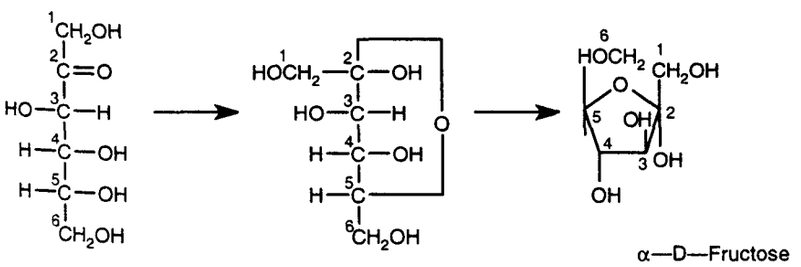


Figure 4-3. Structure of fructose.

3. **Derivatives of glucose.** Glucose can be modified to give three important compounds found in metabolism (Figure 4-4).
   a. **D-gluconic acid** is formed by the oxidation of the aldehyde at C-1, producing an "aldonic" acid. The phosphorylated

IN A NUTSHELL

- *D-gluconic acid → hexose monophosphate shunt*
- *D-glucuronic acid → proteoglycans*
- *D-glucosamine → glycoproteins, proteoglycans, and glycolipids*

form of gluconic acid is an important intermediate in the hexose monophosphate shunt.

b. **D-glucuronic acid** is formed by oxidation of the alcohol at C-6, producing a "uronic" acid. Ascorbic acid (vitamin C) is synthesized from glucuronic acid. The activated carrier of glucuronic acid (UDP-glucuronic acid) is used in the synthesis of proteoglycans.

c. **D-glucosamine** is formed by the substitution of an amino group for the hydroxyl group of C-2, resulting in glucosamine. Glucosamine and galactosamine are important structural components of glycoproteins and proteoglycans.

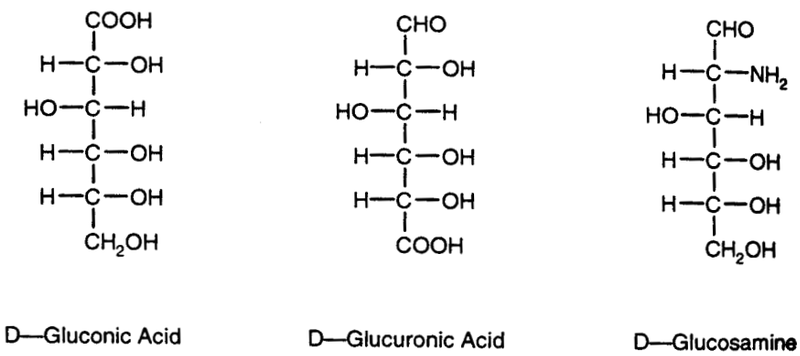


Figure 4-4. Derivatives of D-glucose.

B. **Disaccharides** are formed when two monosaccharides are connected by a glycosidic linkage. The anomeric carbon of one monosaccharide is usually linked to a hydroxyl group on the second monosaccharide. The glycosidic bond is designated as α or β, depending on the configuration of the anomeric carbon in the linkage. The two most important disaccharides in human biochemistry are lactose and sucrose (Figure 4-5).

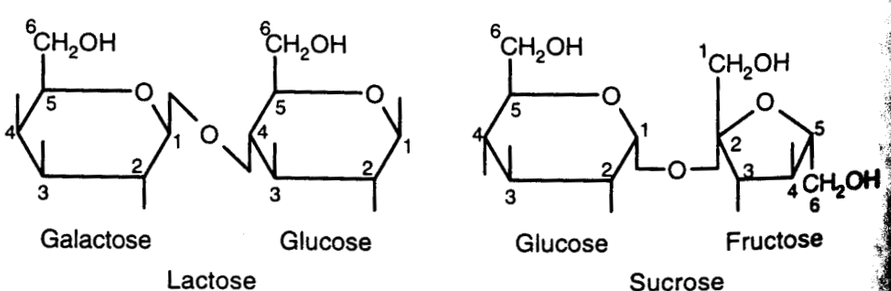


Figure 4-5. Structures of lactose and sucrose.

1. **Lactose,** also known as milk sugar, is a disaccharide of galactose linked through its anomeric carbon to the 4-hydroxyl group of glucose. Thus, the monosaccharides are joined by a β-1,4 glycosidic linkage. Lactose is hydrolyzed to glucose and galactose by **lactase,** an enzyme found in the brush border membrane of the small intestine.
2. **Sucrose**, or table sugar, is a disaccharide of glucose and fructose linked together through their anomeric carbon atoms in an α-1,2 linkage. Sucrose is hydrolyzed to glucose and fructose in the small intestine by the enzyme **sucrase.**

Oligosaccharides are arbitrarily defined as having between two and ten monosaccharides linked by glycosidic bonds. They are found in mucoproteins and glycolipids.

Polysaccharides are arbitrarily defined as having more than ten monosaccharide units. They serve as structural components of cells and the extracellular matrix, as storage forms for monosaccharides, and as dietary fiber. The most common polysaccharides are starch, glycogen, cellulose, and proteoglycans.

1. **Starch** and **glycogen** are both storage forms for glucose. Starch, the major plant polysaccharide, is composed of **amylose** and **amylopectin.** Amylose is a long, unbranched chain of glucose units linked by α-1,4 bonds. Amylopectin has a similar structure, but it also contains branches, where the monosaccharide at the branch point is linked to a monosaccharide in the straight chain by an α-1,6 glycosidic bond. Glycogen, the major animal polysaccharide, is structurally similar to amylopectin, except it is more highly branched.
2. **Cellulose** is a linear plant polysaccharide composed of glucose units linked together by β-1,4 glycosidic bonds. Cellulose is not digested by humans because there is no intestinal enzyme for hydrolyzing glucose units linked by β-1,4 glycosidic bonds. Therefore, cellulose acts as a dietary fiber, providing "roughage" in the diet.
3. **Proteoglycans,** also known as **mucopolysaccharides**, are major structural components of the extracellular matrix. Examples are hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratan sulfate. These compounds are linear carbohydrate polymers with more than one kind of monosaccharide, linked covalently to a protein core. The carbohydrate portions of proteoglycans are known as **glycosaminoglycans (GAGs)**. GAGs contain repeating disaccharides, usually a hexosamine (glucosamine or galactosamine) and a uronic acid (either glucuronic acid or iduronic acid). The

CLINICAL CORRELATE

Primary lactose intolerance is caused by a hereditary deficiency of lactase, most commonly found in persons of Asian and African descent. Secondary lactose intolerance can be precipitated at any age by gastrointestinal disturbances such as celiac sprue, colitis, or viral-induced damage to intestinal mucosa. Common symptoms of lactose intolerance include vomiting, bloating, explosive and watery diarrhea, cramps, and dehydration. The symptoms can be attributed to bacterial fermentation of lactose to a mixture of CH_4, H_2, and small organic acids. The acids are osmotically active and result in the movement of water into the intestinal lumen.

NOTE

Heparan is a proteoglycan found in the extracellular matrix and is associated with the cell surface of skin and fibroblasts; heparin is a proteoglycan found mainly in mast cell granules and functions primarily as an anticoagulant.

BRIDGE TO MICROBIOLOGY

Staphylococci can invade connective tissue because they possess hyaluronidase, an enzyme that degrades hyaluronic acid.

polymers are usually sulfated and the hexosamines are acylated. The exception is hyaluronic acid; it is not linked to a protein core, it is not sulfated, and it is the only member of this class of compounds found in bacterial sources. The proteoglycans are highly asymmetrical and have a high density of negative charge, allowing them to absorb large quantities of water and form viscous solutions. These physical properties account for their ability to serve as excellent shock absorbers and lubricants.

4. **Glycoproteins** and mucoproteins also contain carbohydrate covalently linked to protein, but there are no repeating disaccharides and the carbohydrate portion usually contains fewer than 20 monosaccharides. Glycoproteins are found in connective tissue (collagen), in plasma (plasma proteins and some peptide hormones), on cell surfaces as antigens (i.e. blood groups), and as components of mucus. There are three types of linkages between protein and carbohydrate. The amino acid side chains involved in the protein-carbohydrate linkage are:
   a. **Asparagine** (N-linkage) is found in plasma and cell surface proteins.
   b. **Serine** (O-linkage) is found in mucous and connective tissue proteins.
   c. **5-Hydroxylysine** (O-linkage) is found in collagen.

   Glycoprotein synthesis starts in the endoplasmic reticulum and is completed in the Golgi. One of the last steps in glycoprotein synthesis is putting "zip codes" on proteins that are targeted for a particular destination. For example, glycoproteins that are targeted for lysosomes have mannose-6-phosphate in their carbohydrate chains. Receptors on lysosomes recognize this signal and take up these proteins.

GLUCOSE ENTRY AND TRAPPING IN CELLS

Circulating glucose supplies all cells with a source of energy. The entry of glucose into cells is mediated by a group of carrier proteins (GLUT proteins) that span the plasma membrane. Net transport across the membrane is ensured by coupling glucose transport with phosphorylation, a process that keeps the intracellular glucose concentration low and continues to shift the equilibrium toward glucose uptake by cells (Figure 4-6). The membrane is highly impermeable to the phosphorylated compounds. Therefore, phosphorylation renders glucose transport irreversible.

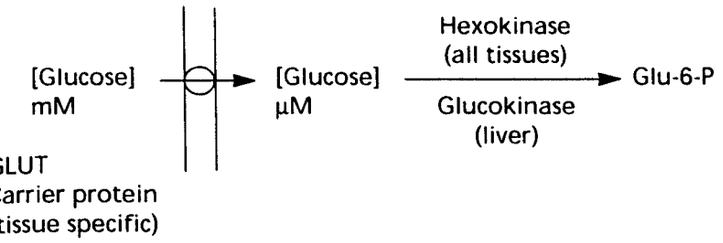


Figure 4-6. Glucose transport and trapping in cells.

Glucose carriers (GLUT proteins). A family of homologous proteins, known as GLUT proteins, are responsible for transporting glucose into the cell. These proteins differ in tissue specificity, their affinity for glucose, and the maximum rate at which they can transport glucose across the plasma membrane.

1. Skeletal muscle and adipose tissue respond to insulin by increasing their uptake of glucose. These tissues contain GLUT-4, a glucose carrier with spare copies in the Golgi membrane. Stimulation by insulin brings the spare carriers into the plasma membrane.
2. Liver has a high density of GLUT-2, a transporter that has a low affinity (high K_m) for glucose and is not saturated by the increased concentration of glucose in the portal circulation following a high carbohydrate meal.
3. The brush-border membrane of intestinal and kidney cells contain S-GLUT, a carrier that requires sodium for glucose transport.

Glucose phosphorylation and trapping. The phosphorylation of glucose in most cells is catalyzed by **hexokinase**. Liver contains **glucokinase**, an isozyme that has a much lower affinity (higher K_m) for glucose and is never saturated under physiological conditions. Therefore, the properties of glucokinase, in concert with GLUT-2, allow the liver to reduce the concentration of glucose in the portal circulation following a meal.

MNEMONIC

***Hexokinase** places a **hex** on glucose, with ↓K_m and ↓V_{max}. Hexokinase is inhibited by ↑[G6P].*

***Glucokinase** is a **glu**tton for glucose, with ↑K_m and ↑V_{max}; it does not seem to be affected by the amount of glucose-6-phosphate.*

GLYCOLYSIS

Glycolysis, also known as the Embden-Myerhof pathway, is the central pathway of glucose metabolism. It occurs in the cytosol of all cells. Glycolysis is defined as the pathway that converts glucose to pyruvate. For each mol of glucose converted to **pyruvate**, 2 mols of ATP are consumed and 4 mols are generated, with a net production of 2 mols of ATP per mol of glucose (discussed below). In the presence of well-oxygenated mitochondria, pyruvate can be completely oxidized to CO_2 and H_2O, resulting in a total of 36-38 mols ATP per mol of glucose. Under anaerobic conditions, however, pyruvate is

converted to **lactate**. The formation of lactate provides a mechanism for regenerating NAD^+ from NADH, a condition that is essential for glycolysis to continue. Anaerobic glycolysis plays an important role in ATP production under conditions where O_2 is limited, e.g., in exercising skeletal muscle or in cells that lack mitochondria, such as red blood cells.

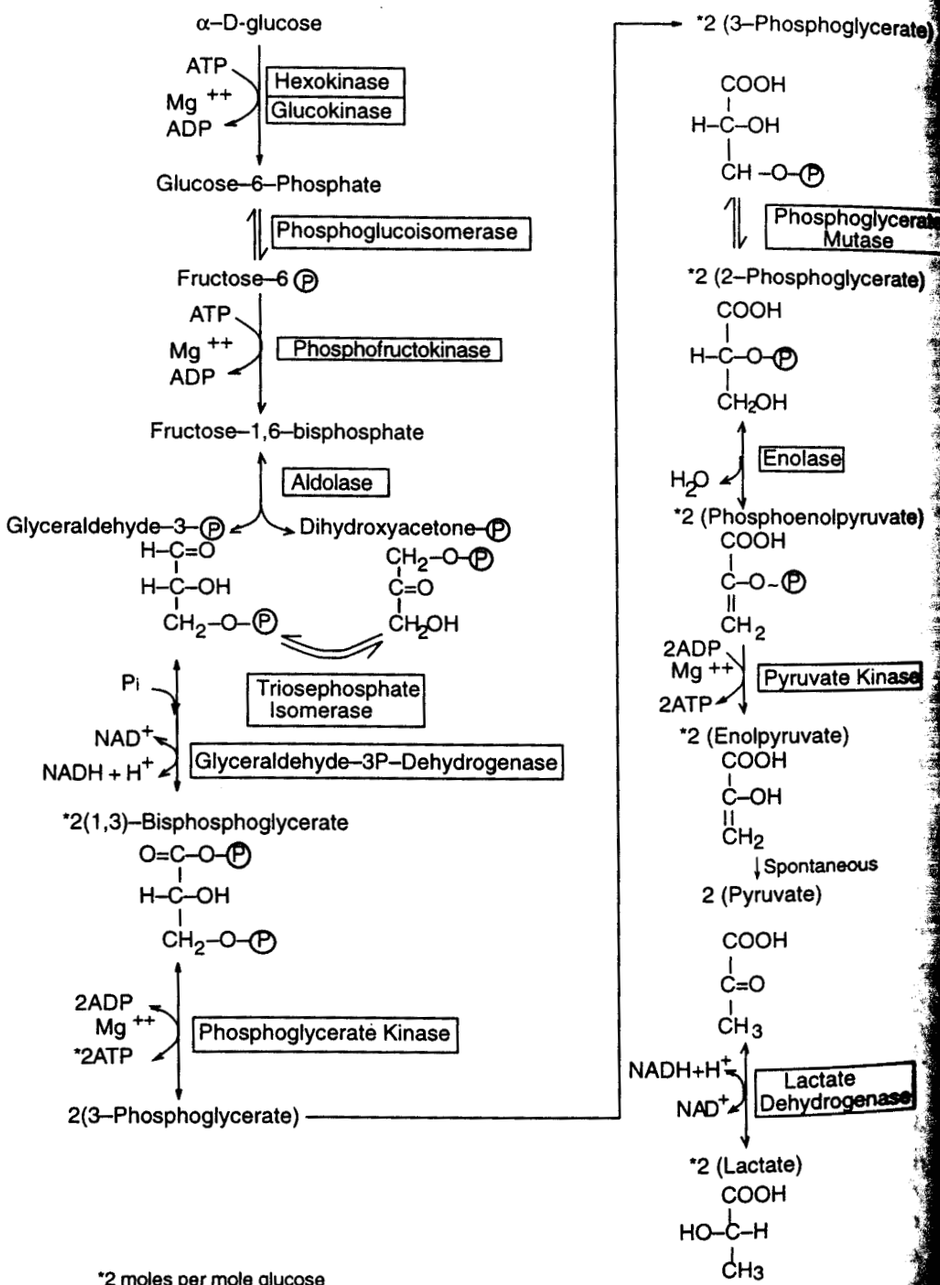


Figure 4-7. Glycolysis.

unctions. Glycolysis generates ATP and provides intermediates iat can be used in other pathways. For example, both glucose-6-hosphate and pyruvate act as branch points in metabolism ecause they are substrates for enzymes in other pathways.

athway and enzymes. The pathway of glycolysis is shown in gure 4-7. It can be divided into two stages. The first stage equires the expenditure of ATP, while the second stage results in ie net production of ATP.

1. **Stage 1 of glycolysis.** Glucose is converted to fructose-1,6-P_2 through three sequential reactions. ATP is required for the addition of phosphate groups to both ends of the monosaccharide. The two key enzymes in stage 1 are hexokinase and phosphofructokinase-1 (PFK-1).
   a. **Hexokinase** (or glucokinase in liver) uses ATP to convert glucose to glucose-6-phosphate. In the next step, glucose-6-phosphate is isomerized to fructose-6-phosphate.
   b. **PFK-1** uses ATP to add phosphate to the C-1 of fructose-6-phosphate, with the formation of fructose-1,6-bisphosphate. This reaction is the **rate-limiting step in glycolysis.** PFK-1 is therefore the primary site of regulation in glycolysis.
   c. **Phosphofructokinase-1** and **phosphofructokinase-2** both use fructose-6-phosphate as substrate. However, the two different enzymes have distinctly different functions. The product of the PFK-1 reaction is fructose-1,6-P_2, an intermediate in glycolysis. The product of the PFK-2 reaction is fructose-2,6-P_2. The only known function of this compound is to act as an allosteric effector. It activates glycolysis and inhibits the opposing pathway of gluconeogenesis. The relationship between PFK-1 and PFK-2 is shown in Figure 4-8.

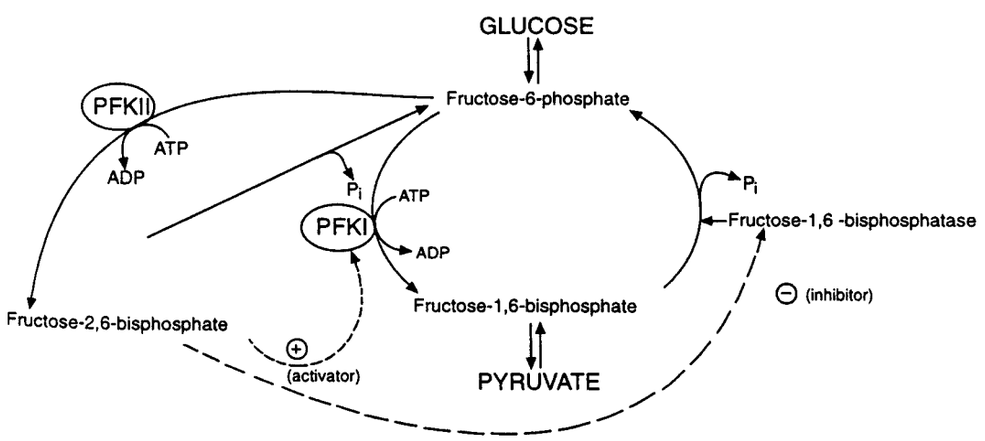


Figure 4-8. Relationship between PFK-1 and PFK-2.

2. **Stage 2 of glycolysis.** The function of the second stage is to produce ATP. It begins with the cleavage of fructose-1,6-bisphosphate by aldolase into two phosphorylated trioses (dihydroxyacetone phosphate and glyceraldehyde-3-phosphate). These two compounds are interconvertible through the action of triosephosphate isomerase. This reaction allows both trioses to proceed by a common pathway. Thus, beginning from this step in glycolysis, each mol of glucose can be considered to produce two mols of glyceraldehyde-3-phosphate and all subsequent intermediates. The remaining steps in the pathway are concerned with generating intermediates having high-energy phosphate groups that can be transferred to ADP with the formation of ATP. Two intermediates, **1,3-bisphosphoglycerate** and **phosphoenolpyruvate**, have enough energy to drive the synthesis of ATP. There are three important enzymes in stage 2 of glycolysis: glyceraldehyde-3-phosphate dehydrogenase (G-3-P DH), 3-phosphoglycerate kinase, and pyruvate kinase. Under anaerobic conditions, lactate dehydrogenase is an also important enzyme in glycolysis.
   a. **Glyceraldehyde-3-phosphate dehydrogenase** catalyzes a reversible reaction that occurs in two steps. First, the aldehyde group is oxidized to a carboxylic acid, with NAD^+ being reduced to NADH. (Note that this is the only oxidation reaction in glycolysis.) Second, inorganic phosphate is covalently linked to the carboxyl group, forming 1,3-bisphosphoglycerate. The phosphate bond created in this reaction is a high-energy bond, with a ΔG° of –11.8 kcal/mol (only 7.3 kcal/mol are needed to convert ADP to ATP).
   b. **3-Phosphoglycerate kinase** transfers the high-energy phosphate group from 1,3-bisphosphoglycerate to ADP, producing ATP and 3-phosphoglycerate. Thus, 3-phosphoglycerate kinase is one of the ATP-producing enzymes in glycolysis. At this point, the energy consumed in stage 1 has been replaced. In the next two reactions, the phosphate group of 3-phosphoglycerate is rearranged to form another high-energy bond. **Phosphoglycerate mutase** moves the phosphate group from carbon-3 to carbon-2, forming 2-phosphoglycerate. **Enolase** then dehydrates 2-phosphoglycerate to form phosphoenolpyruvate, a high-energy compound with a ΔG° of –14.8 kcal/mol.
   c. **Pyruvate kinase** catalyzes the last reaction in glycolysis. The phosphate group from phosphoenolpyruvate is transferred to ADP with the formation of ATP and pyruvate. Thus, the pathway results in a net of two mols of ATP per mol of glu

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Intermediate	*Enzyme*	*Product*
1,3-bisphosphoglycerate	*Phosphoglycerate kinase*	*ATP*
Phosphoenolpyruvate	*Pyruvate kinase (irreversible)*	*ATP*
Glyceraldehyde-3-phosphate	*G-3P dehydrogenase*	*NADH*

cose. This reaction is irreversible, and pyruvate kinase is a secondary site for regulation of glycolysis.

d. **Lactate dehydrogenase** participates in glycolysis only under anaerobic conditions. Lactate dehydrogenase reduces pyruvate to lactate in a reaction that uses NADH and regenerates NAD^+. In cells with well-oxygenated mitochondria, lactate does not form in significant amounts. However, when oxygenation is poor, as in heavily exercising muscle, shock, or cardiopulmonary arrest, both the citric acid cycle and oxidative phosphorylation become relatively inactive, and most of the cellular ATP is generated by glycolysis. Since glycolysis requires NAD^+ (Step "a" above), the formation of lactate serves as a mechanism for regenerating NAD^+ so that glycolysis can continue. In states of prolonged anoxia, large amounts of lactate accumulate. The lactate is transported to the liver where it can be used to resynthesize glucose.

Regulation of glycolysis. There are three steps in glycolysis that are regulated: The primary site of regulation is PFK-1, the enzyme that catalyzes the slowest step in the pathway. Secondary sites of regulation are hexokinase/glucokinase and pyruvate kinase. The regulatory properties of these enzymes are summarized in Table 4-2. PFK-1 is inhibited by ATP and citrate, molecules that indicate a high-energy status. When AMP accumulates, signaling the need for ATP, PFK-1 is activated. Pyruvate kinase is also inhibited by molecules that indicate a high energy state (ATP and acetyl-CoA). When fructose-1,6-bisphosphate accumulates, it feeds forward and activates pyruvate kinase. In liver, the most important activator of glycolysis is fructose-2,6-bisphosphate, a compound whose concentration is increased by insulin and decreased by glucagon.

ızyme	Mode of Regulation	Effect
exokinase	Allosteric	Inhibition: glucose-6-P[a]
lucokinase[b]	Enzyme synthesis	Induced by insulin, not inhibited by glucose-6-P
K-1	Allosteric	Activation: AMP, fructose-2,6-P_2[b] Inhibition: ATP, citrate
ruvate kinase	Allosteric	Activation: fructose-1,6-P_2 Inhibition: ATP, acetyl-CoA, alanine[b]
	Covalent[b]	Inhibited by phosphorylation[b]

Accumulates when ATP is high
Effects are liver-specific

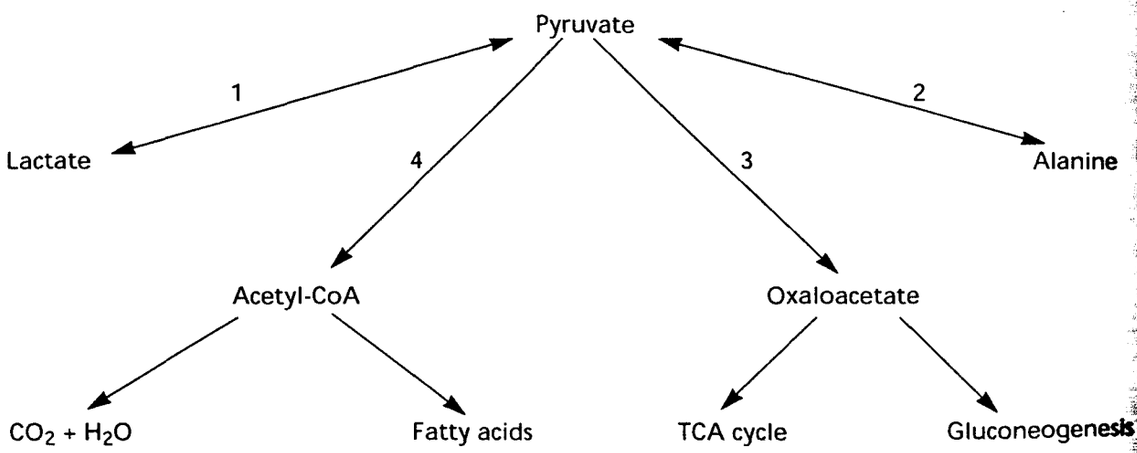
Table 4-2. Regulation of glycolysis.

CLINICAL CORRELATE

Hemolytic anemia is caused by excessive destruction of red blood cells. A deficiency in pyruvate kinase is a common enzyme deficiency that results in premature lysis of the red blood cell. The red blood cell has no mitochondria and is totally dependent on glycolysis for ATP. A deficiency in pyruvate kinase, therefore, markedly decreases the ability to synthesize ATP. Maintenance of the biconcave shape of the red blood cell is dependent on ATP. Loss of shape signals uptake and turnover by the spleen. In addition, decreased ion pumping by Na^+/K^+ ATPase results in loss of ion balance and causes osmotic fragility, leading to swelling and lysis.

AN

D. **Fate(s) of pyruvate.** Pyruvate, the end product of glycolysis, plays a central role in metabolism as shown in Figure 4-9. Pyruvate can be reversibly converted to lactate and to alanine. Both of these reactions occur in the cytosol. Lactate is generated under anaerobic conditions by lactate dehydrogenase. Alanine is formed mainly in skeletal muscle, where it serves as a vehicle for transferring amino groups from muscle to liver, where they are then incorporated into to urea (discussed in Chapter 6). Pyruvate also undergoes two irreversible reactions, both occurring in the mitochondria. It can be carboxylated to oxaloacetate, which can replenish TCA cycle intermediates or be used for gluconeogenesis. Pyruvate can also be converted to acetyl-CoA, which can be used as a building block for fatty acids, or it can be oxidized to CO_2 and H_2O by the TCA cycle and oxidative phosphorylation to generate ATP.



Enzymes 1. Lactate dehydrogenase; 2. Transaminase;
3. Pyruvate carboxylase; 4. Pyruvate dehydrogenase

Figure 4-9. Metabolic fates of pyruvate.

1. **Pyruvate dehydrogenase (PDH).** PDH is a multienzyme complex that converts pyruvate to acetyl-CoA by oxidative decarboxylation. This reaction is **irreversible** and there is no enzyme in humans that will reverse the reaction. The overall reaction catalyzed by PDH is:

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{Acetyl-CoA} + CO_2 + \text{NADH}$$

The enzyme complex is located in the mitochondria and consists of **three distinct enzyme activities** (decarboxylase, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase).

Five coenzymes are required (thiamine pyrophosphate, lipoic acid, Coenzyme A, FAD, and NAD^+). The reaction occurs in several steps as shown in Figure 4-10. In the first step, the decarboxylase (E_1) and its cofactor, thiamine pyrophosphate (TPP) release CO_2. The C_2 fragment that remains is oxidized by lipoic acid, transferred to CoA, and finally released as acetyl-CoA. These reactions are catalyzed by dihydrolipoyl transacetylase (E_2). The last step, catalyzed dihydrolipoyl dehydrogenase (E_3) and its cofactors FAD and NAD^+, regenerates the initial oxidized form of lipoic acid. The cofactors TPP, lipoic acid, and FAD never leave the enzyme complex.

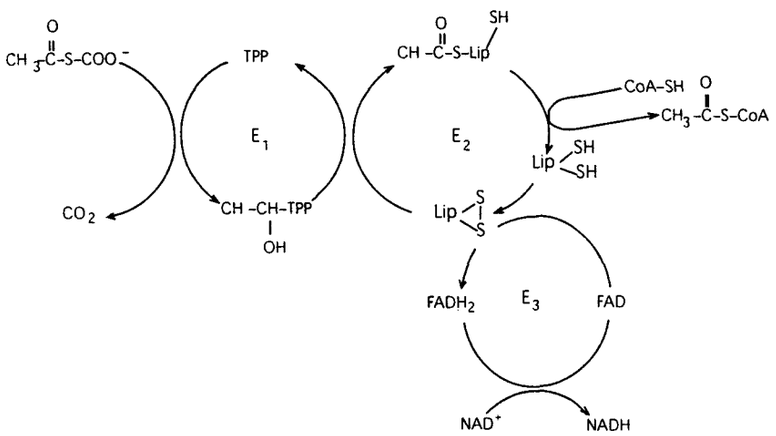


Figure 4-10. The pyruvate dehydrogenase complex.

2. **Regulation of pyruvate dehydrogenase.** The activity of PDH is dependent on the energy state of the cell as reflected by the levels of acetyl-CoA, ATP, and NADH. It is inhibited by ADP, CoA, and NAD^+. Acetyl-CoA and NADH are powerful inhibitors of pyruvate dehydrogenase activity. As shown in Figure 4-11, PDH undergoes phosphorylation/dephosphorylation. A specific protein kinase and protein phosphatase are associated with the multienzyme complex. The kinase is activated by acetyl-CoA and NADH. The phosphatase is activated by insulin.

   The regulation of pyruvate dehydrogenase is important to fuel conservation. For example, the oxidation of carbohydrate, fat, and proteins all produce acetyl-CoA and NADH (as seen in Figure 3-1 in the Bioenergetics chapter). Thus, when fatty acids are being oxidized, carbohydrate (pyruvate) and protein are conserved.

3. **Homology of pyruvate dehydrogenase with other enzymes.** Two other multienzyme complexes in human metabolism have

similar structures to pyruvate dehydrogenase. These are α-ketoglutarate dehydrogenase (in the TCA cycle) and branched-chain keto acid dehydrogenase (in amino acid catabolism). All three of these multienzyme complexes catalyze the oxidative decarboxylation of α-ketoacids. They all have E_1, E_2, and E_3 enzyme activities that require the same five coenzymes.

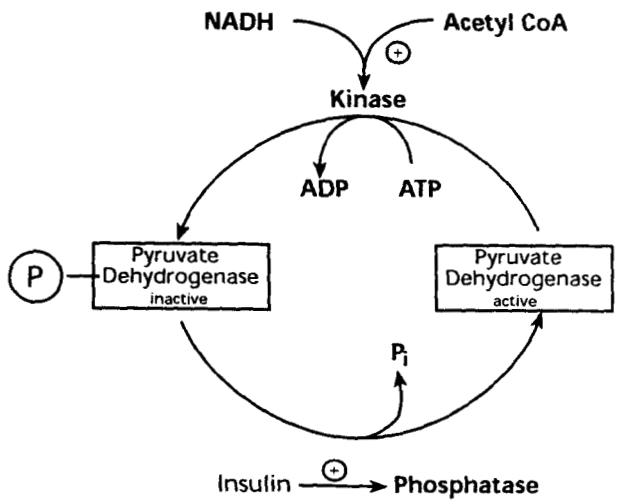


Figure 4-11. Regulation of PDH activity.

E. **Energetics of glycolysis.** The amount of ATP derived from glucose oxidation is dependent on the availability of oxygen.

1. **Anaerobic glycolysis.** When oxygen is limited, glycolysis can be summarized by the equation given below. For each mol of glucose consumed, two mols of lactate and two mols of ATP are produced. There is no net accumulation of NADH.

$$\text{Glucose} + 2\ \text{ADP} + 2\ P_i \rightarrow 2\ \text{lactate} + 2\ \text{ATP}$$

2. **Aerobic glycolysis.** When oxygen is plentiful, glycolysis can be summarized as follows:

$$\text{Glucose} + 2\ NAD^+ + 2\ \text{ADP} + 2\ P_i \rightarrow 2\ \text{pyruvate} + 2\ \text{ATP} + 2\ \text{NADH}$$

For each mol of glucose consumed, 2 mols each of pyruvate, ATP, and NADH are produced. The NADH can undergo oxidative phosphorylation, producing either 2 or 3 mols of ATP, depending on how electrons in the cytosolic NADH are transported into the mitochondria. Use of the malate shuttle results in 3 mols of ATP per NADH, while use of the α-glycerol-phosphate shuttle results in 2 mols of ATP per NADH. Additionally, each mol of pyruvate can be transported into the mitochondria and oxidized to CO_2 and H_2O by the TCA cycle and oxidative phosphorylation, resulting in 15 mols of high-

CLINICAL CORELATE

Lactic acidosis arising from a deficiency in pyruvate dehydrogenase (PDH) may be an acquired or an inherited condition. The inherited form frequently presents in the neonatal period with vomiting, hypotonia, neurologic deficits, and persistent acidosis. The accumulation of pyruvate behind the metabolic block pushes the equilibrium of the reversible lactate dehydrogenase and alanine transaminase reactions, thus accumulating lactate and alanine. Acquired PDH deficiency is frequently seen in chronic alcoholics who have a thiamine deficiency. The elevated fat content of the diet given as treatment results in an excess of acetyl-CoA, which is converted to ketones. The ketones can be used as fuel by the brain, thereby bypassing the PDH reaction.

energy phosphate (ATP or GTP) per mol of pyruvate. The enzymes involved in generating ATP (or GTP) from glucose are summarized in Table 4-3.

Poisons of glycolysis. Many compounds shut down the glycolytic pathway by inhibiting one or more of the enzymes. Note, in particula, fluoride.

1. **Fluoride** inhibits enolase by complexing with 2-phosphoglycerate and Mg^{2+}, making the substrate unavailable.

hway	Enzyme	Product	Method of energy generation	ATP/glucose
TOSOL)				
COLYSIS				
	1. Glyceraldehyde-3-P dehydrogenase	2 NADH	Oxidative-phosphorylation	4-6[a]
	2 Phosphoglycerate kinase	2 ATP	Substrate-level phosphorylation	2
	3. Pyruvate kinase	2 ATP	Substrate-level phosphorylation	2
	ATP produced/glucose			8-10[a]
	ATP consumed/glucose (hexokinase and phosphofructokinase)			−2
	Net ATP produced/glucose			6-8[a]
ITOCHONDRIA)				
H & TCA CYCLE				
	1. Pyruvate dehydrogenase	2 NADH	Oxidative-phosphorylation	6
	2. Isocitrate dehydrogenase	2 NADH	Oxidative-phosphorylation	6
	3. α-Ketoglutarate dehydrogenase	2 NADH	Oxidative-phosphorylation	6
	4. Succinate thiokinase	2 GTP	Substrate-level phosphorylation	2
	5. Succinate dehydrogenase	2 $FADH_2$	Oxidative-phosphorylation	4
	6. Malate dehydrogenase	2 NADH	Oxidative-phosphorylation	6
Net ATP produced/glucose				30
tal ATP per glucose (aerobic oxidation) = 36-38[a]				
tal ATP per glucose (anaerobic oxidation) = 2 (Reactions 2 and 3 of glycolysis)				
ansfer of electrons in cystolic NADH via malate shuttle produces mitochondrial NADH and 3 ATP, hereas transfer via α-glycerol-P shuttle produces $FADH_2$ and 2 ATP.				

Table 4-3. Energetics of aerobic oxidation of glucose.

GLUCONEOGENESIS

Gluconeogenesis is the pathway for *de novo* synthesis of glucose from C_3 and C_4 precursors. This pathway is distinct from glycogenolysis, which gives rise to preformed glucose. Gluconeogenesis occurs mainly in the liver and kidney, with a small amount occurring in the epithelium of the small intestine. The pathway requires both mitochondrial and cytosolic enzymes.

A. **Function.** The role of gluconeogenesis in homeostasis is to maintain proper blood glucose levels and to provide glucose for the body. The brain, central nervous system, and red blood cells are dependent on glucose for all or most of their energy. When fasting persists for more than 12 to 24 hours, liver glycogen stores are exhausted and gluconeogenesis provides glucose for these tissues. The primary precursors for *de novo* glucose synthesis are lactate (from red blood cells and anaerobic muscle), glycerol (from triacylglycerol degradation in adipocytes), and amino acids (from protein degradation in skeletal muscle).

CLINICAL CORRELATE

A deficiency in any of the four key enzymes used in gluconeogenesis will result in hypoglycemia.

B. **Pathway and key enzymes.** Gluconeogenesis uses the seven enzymes in the glycolytic pathway that catalyze reversible reactions. Additionally, there are four enzymes unique to gluconeogenesis that are required to bypass the three irreversible reactions in glycolysis (Figure 4-12).

Each of the four enzymes unique to gluconeogenesis also catalyzes an irreversible reaction, but in the opposite direction from the corresponding enzyme in glycolysis. In order to bypass the pyruvate kinase reaction, two enzymes are required, pyruvate carboxylase and phosphoenolpyruvate carboxykinase (PEPCK).

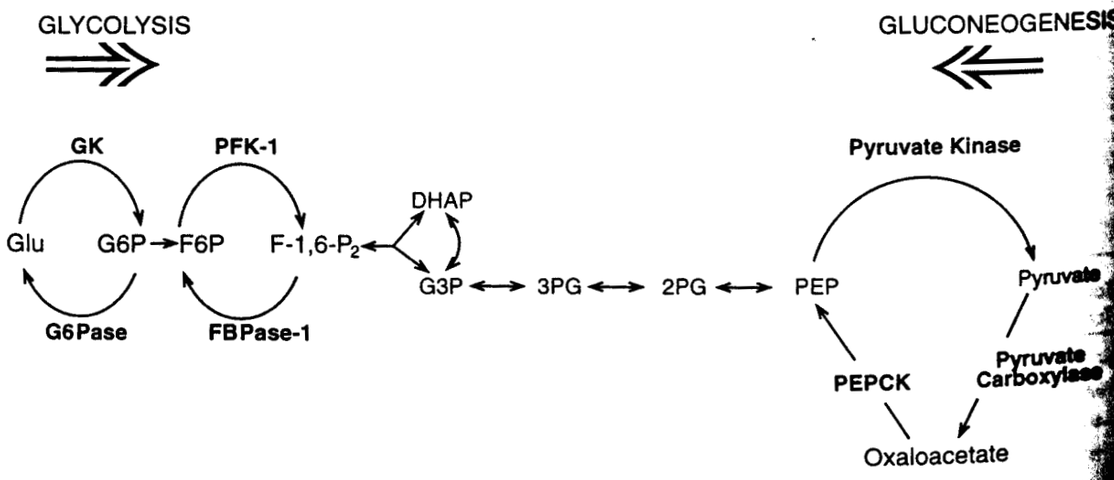


Figure 4-12. Gluconeogenesis and glycolysis.

1. **Pyruvate carboxylase,** an enzyme located in the mitochondria, catalyzes the carboxylation of pyruvate to oxaloacetate, the first step in gluconeogenesis (Figure 4-13). Acetyl-CoA is an allosteric activator that must be present for the enzyme to function. Biotin serves as a carrier of the "activated carboxyl" group derived from bicarbonate. ATP hydrolysis to ADP and P_i provides the energy needed to generate the "activated carboxyl" group.

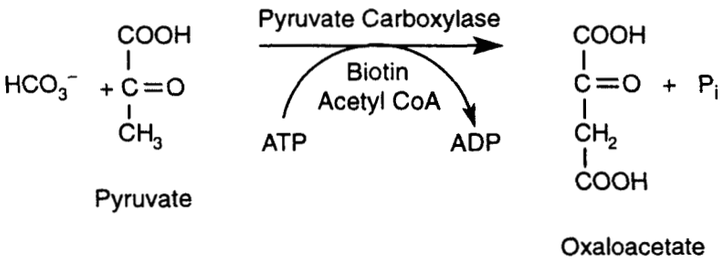
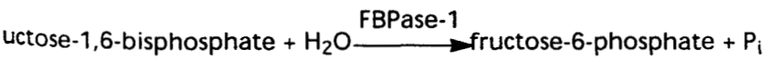


Figure 4-13. The pyruvate carboxylase reaction.

2. **Phosphoenolpyruvate carboxykinase (PEPCK)** catalyzes the second step in gluconeogenesis (Figure 4-14). PEPCK is a cytosolic enzyme that phosphorylates and decarboxylates oxaloacetate to form phosphoenolpyruvate. The phosphate group is derived from GTP, which is energetically equivalent to ATP. Thus, in order to bypass the irreversible pyruvate kinase reaction, two enzymes are required and the equivalent of two mols of ATP are consumed in converting pyruvate to phosphoenolpyruvate.
3. **Fructose-1,6-bisphosphatase (FBPase-1)** catalyzes the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate and inorganic phosphate. This reaction bypasses the irreversible step in glycolysis catalyzed by PFK-1.



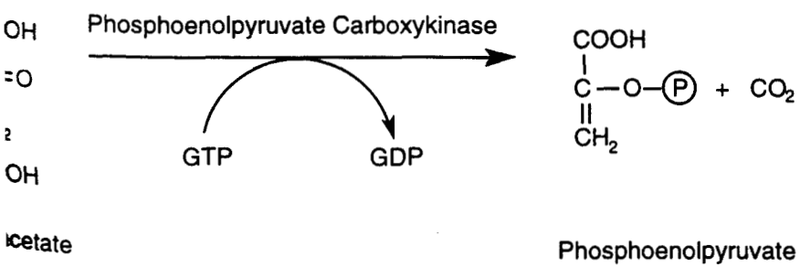


Figure 4-14. The PEPCK reaction.

FBPase-1 is a cytosolic enzyme activated by ATP and citrate and inhibited by AMP and fructose-2,6-bisphosphate. These same compounds alter the activity of the opposing enzyme, PFK-1, but in the opposite direction (see Table 4-2). Thus, glycolysis is inhibited when gluconeogenesis is occurring, and vice-versa.

4. **Glucose-6-phosphatase (G6Pase)** catalyzes the last step in gluconeogenesis by removing the phosphate from glucose-6-phosphate and releasing free glucose.

$$\text{Glucose-6-phosphate} + H_2O \xrightarrow{\text{G6Pase}} \text{glucose} + P_i$$

This reaction bypasses the irreversible hexokinase step in glycolysis. G6Pase is associated with the endoplasmic reticulum, and is found only in liver, kidney, and intestinal epithelium. The absence of G6Pase in skeletal muscle accounts for the fact that muscle glycogen cannot serve as a source of blood glucose.

CLINICAL CORRELATE

A deficiency in glucose-6-phosphatase results in von Gierke's disease, which is characterized by low serum glucose and high serum lactate and pyruvate due to the compensatory increase in liver glycolysis.

C. **Overall reaction of gluconeogenesis.** The conversion of pyruvate to glucose via gluconeogenesis is shown below. For every mol of glucose synthesized, six ATP equivalents are required. Four ATP equivalents are required to convert pyruvate to phosphoenolpyruvate, and two are required to reverse the 3-phosphoglycerate kinase step in glycolysis. The NADH is used to reverse the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase.

$$2 \text{ pyruvate} + 4 \text{ ATP} + 2 \text{ GTP} + 2 \text{ NADH} \rightarrow \text{glucose} + 4 \text{ ADP} + 2 \text{ GDP} + 6 P_i + 2 \text{ NAD}^+$$

The ATP required for gluconeogenesis is derived from the oxidation of fatty acids. It should be noted that the reaction may begin with lactate rather than pyruvate, since the lactate dehydrogenase reaction is reversible.

D. **Precursors for gluconeogenesis.** Any compound that can be converted to an intermediate in glycolysis or the citric acid cycle can serve as a precursor for gluconeogenesis.

1. **Lactate.** The function of the **Cori cycle** is to conserve glucose by recycling lactate formed from anaerobic glycolysis in red blood cells and skeletal muscle. Lactate is released from these tissues into the blood and is taken up by the liver. It is then reconverted to glucose via gluconeogenesis to provide more glucose for energy. The recycling process is illustrated in Figure 4-15.
2. **Alanine.** Skeletal muscle releases large amounts of alanine formed by the transamination of pyruvate. The alanine is taken up by the liver and is reconverted to pyruvate, which is used for glucose synthesis. This recycling is known as the alanine cycle. The alanine cycle provides a means of conserving

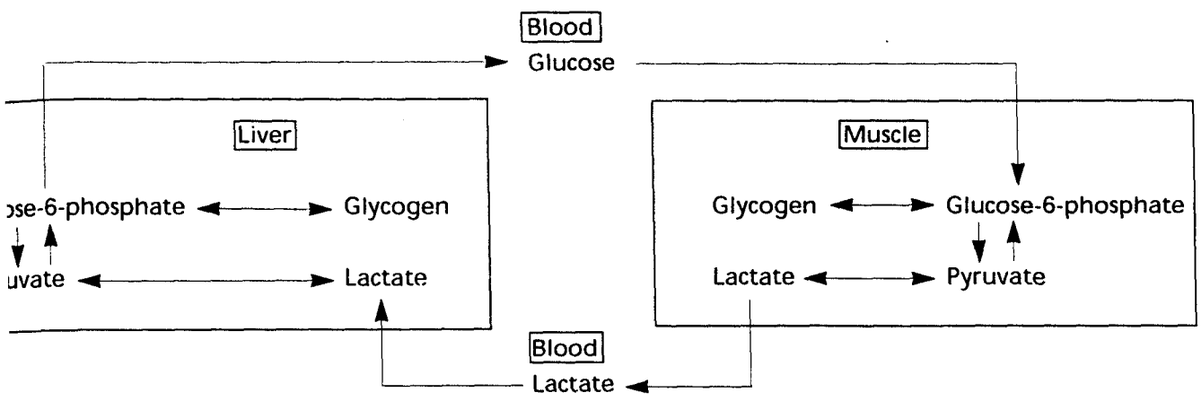


Figure 4-15. The Cori cycle.

carbohydrate. Note that neither the alanine cycle nor the Cori cycle result in the net synthesis of glucose.

3. **Glucogenic amino acids.** All of the common amino acids except lysine and leucine are glucogenic, i.e., they can be degraded to intermediates in either glycolysis or the TCA cycle, and thus can serve as precursors for glucose synthesis. The nitrogen arising from these amino acids is disposed of as urea. Lysine and leucine are strictly ketogenic and cannot be converted to glucose.
4. **Glycerol.** The hydrolysis of triacylglycerols in adipose tissue produces glycerol that is released into the blood. The liver takes up glycerol and converts it to glycerol-3 phosphate, which can be oxidized to dihydroxyacetone phosphate, a gluconeogenic intermediate.
5. **Odd-numbered fatty acids.** The oxidation of odd-numbered fatty acids produces one molecule of propionyl-CoA from the ω-end. Propionyl-CoA can be converted to succinyl-CoA, an intermediate in the TCA cycle, and then to glucose. In contrast, degradation of even-numbered fatty acids produces only acetyl-CoA, which is not a precursor of glucose.
6. **Fructose.** Fructose (from dietary sucrose) can be phosphorylated to fructose-1-phosphate and cleaved to glyceraldehyde and dihydroxyacetone phosphate (DHAP), a glucogenic intermediate. Glyceraldehyde can be converted to glucogenic intermediates either by reduction to glycerol or by phosphorylation to glyceraldehyde-3-phosphate.
7. **Galactose.** Galactose can be phosphorylated by the liver to galactose-1-phosphate and then converted to glucose-6-phosphate by a series of reactions discussed below.

E. **Regulation of gluconeogenesis.** Gluconeogenesis is tightly regulated in order to maintain blood glucose at a normal level. Regulation is accomplished by three methods: substrate availability, enzymatic control, and hormonal control.

1. **Substrate availability.** Under conditions of fasting, the primary substrates for gluconeogenesis are amino acids derived from protein degradation. The oxidation of fatty acids provides the energy required. All of these processes are coordinated by hormonal control.

2. **Enzymatic control.** The enzymes that are regulated are unique to gluconeogenesis. The opposing enzymes of glycolysis and gluconeogenesis at the irreversible steps in these pathways are regulated reciprocially so that when one is active the other is inactive. This prevents futile cycling of substrate by ensuring that glycolysis and gluconeogenesis are not occurring at the same time.
   a. **Pyruvate carboxylase** is activated by acetyl-CoA. The opposing enzyme in glycolysis, pyruvate kinase, is inhibited by acetyl-CoA.
   b. **Fructose-1,6-bisphosphatase** is activated by ATP and citrate and is inhibited by AMP and fructose-2,6-bisphosphate. The opposing enzyme in glycolysis, PFK-1, is inhibited by ATP and citrate, and is activated by AMP and fructose-2,6-bisphosphate.
   c. **Glucose-6-phosphatase** and the opposing enzyme glucokinase are both regulated by substrate availability. Both enzymes have high K_m values and are not saturated under the conditions in the cell. Therefore, as glucose-6-phosphate and glucose concentrations increase, the activities of glucose-6-phosphatase and glucokinase increase, respectively.

3. **Hormonal control.** The major hormones involved in regulating blood glucose levels are **glucagon** and **insulin**, which have opposing effects. Glucagon promotes glucose synthesis and release into the blood, while insulin promotes glucose uptake and storage. Both of these effects are mediated by intracellular concentrations of cAMP. Cellular cAMP levels increase in response to glucagon and decrease in response to insulin. cAMP activates protein kinase-A. The active form of the protein kinase phosphorylates a subset of enzymes and alters their activities. Under conditions of low blood glucose, the secretion of glucagon initiates several responses that lead to restoring blood glucose levels. Responses to elevated glucagon include:

IN A NUTSHELL

Enzyme	Activators	Inhibitors
Pyruvate carboxylase	*Acetyl-CoA*	*ADP*
Phosphoenol-pyruvate carboxykinase		*ADP*
Fructose-1,6-bisphosphatase	*ATP* *Citrate* *Glucagon* *Epinephrine*	*AMP* *Fructose-2,6-bisphosphate*
Glucose-6-phosphatase		*Glucose*

IN A NUTSHELL

Increased glucagon → increased cAMP → increased protein kinase-A activity → phosphorylation → decreased pyruvate kinase activity

a. **Inhibition of pyruvate kinase.** Phosphorylation of this enzyme leads to its inactivation, thereby decreasing the amount of glucose consumption by glycolysis.
b. **Decreased concentration of fructose-2,6-bisphosphate.** The degradation of fructose-2,6-bisphosphate is stimulated by glucagon. Therefore, its inhibitory effect on FBPase-1 and its stimulatory effect on PFK-1 are both relieved, resulting in increased gluconeogenesis and decreased glycolysis.
c. **Increased synthesis of key enzymes.** The synthesis of PEPCK, FBPase-1, and glucose-6-phosphatase are increased by glucagon. This is a slower response, taking hours rather that minutes or seconds to occur. Synthesis of these enzymes is also induced by glucocorticoids and catecholamines.
d. **Increased protein degradation.** During gluconeogenesis, proteolysis in skeletal muscle is increased, thus providing an increased supply of amino acids for glucose synthesis.
e. **Increased lipolysis**. The release of fatty acids from adipose triglyceride is stimulated by any hormone that increases intracellular cAMP. Fatty acids are taken up by the liver and oxidized to supply the energy required for gluconeogenesis.

CLINICAL CORRELATE

Ethanol-induced hypoglycemia is due to the oxidation of ethanol by the liver, leading to the accumulation of excessive NADH and a deficit of NAD^+. This prevents the oxidation of lactate to pyruvate and α-glycerol-P to dihydroxyacetone phosphate. Gluconeogenesis therefore fails due to inadequate prescursors, resulting in hypoglycemia.

GLYCOGENESIS AND GLYCOGENOLYSIS

Glycogen is a highly branched polymer containing glucose molecules linked by α-1,4 glycosidic bonds, with α-1,6 glycosidic bonds between the two glucose molecules at the branch points. Branching increases the solubility of the molecule and facilitates breakdown of glycogen. At the core of the glycogen particle is the protein glycogenin, which serves as the initial primer in the synthesis of glycogen. Glycogenin is not only the primer for the first glucose molecule, but is also the catalyst for the synthesis of the first 8 glucose residues of the glycogen molecule. The synthesis and degradation of glycogen occur mainly in the liver and skeletal muscle. The liver may store up to 10% of its wet weight as glycogen. All of the synthesis and degradation occurs at the ends of the branch points.

A. **Functions.** Glycogen is the storage form for glucose in animal tissues. The function of glycogen in skeletal muscle differs from that in the liver.

1. **Skeletal muscle** degrades glycogen and rapidly metabolizes glucose via glycolysis. This process generates ATP required for muscle contraction. Thus, the function of glycogen in muscle is to provide energy for contraction. In white ("fast") muscle fibers, glucose is released from glycogen and metabolized by

MNEMONIC

The well-"red" person is slow, but complete: red muscle fibers are slow, but completely oxidize fuel (TCA cycle)

glycolysis, with lactate being the major end product. In red ("slow") muscle fibers, pyruvate is completely oxidized by the TCA cycle and oxidative phosphorylation.

2. **Liver** uses glycogen mainly to regulate blood glucose levels. In response to hypoglycemia, liver glycogen is degraded, and glucose is released into the blood. However, liver glycogen stores become depleted after approximately 12 hours of fasting. In response to hyperglycemia, glucose is removed from the blood and stored as liver glycogen. The pathways of glycogenesis and glycogenolysis are shown in Figure 4-16.

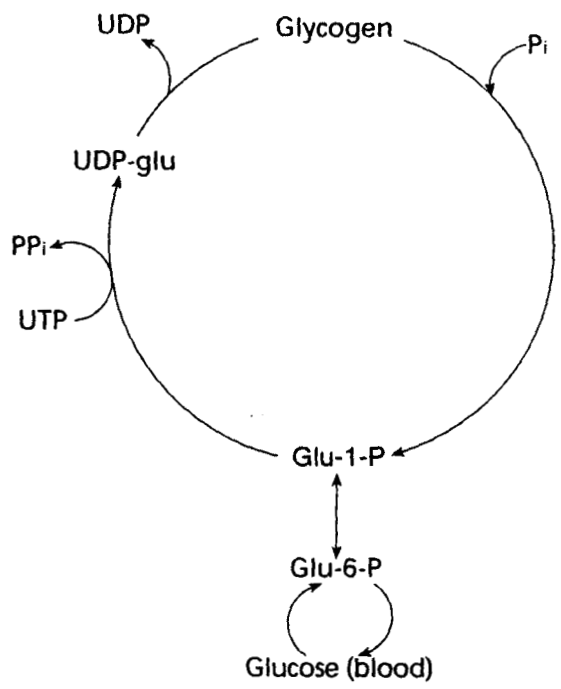


Figure 4-16. Overview of glycogen metabolism.

B. **Key enzymes in glycogenolysis.** Glycogen degradation occurs at the ends of the branches, where glucose-1-P units are sequentially released by **glycogen phosphorylase,** the most important enzyme in glycogenolysis. Total degradation requires an additional "debranching" enzyme system.

1. **Glycogen phosphorylase** cleaves α-1,4 glycosidic bonds by the addition of inorganic phosphate, a process known as "phosphorolysis." This reaction is the rate-limiting step in glycogenolysis and is regulated both allosterically and hormonally. The glycogen phosphorylase reaction is shown below, where n is the number of glucose units in glycogen.

$$(\text{Glucose})_n \xrightarrow[P_i]{\text{glycogen phosphorylase}} (\text{Glucose})_{n-1} + \text{Glucose-1-P}$$

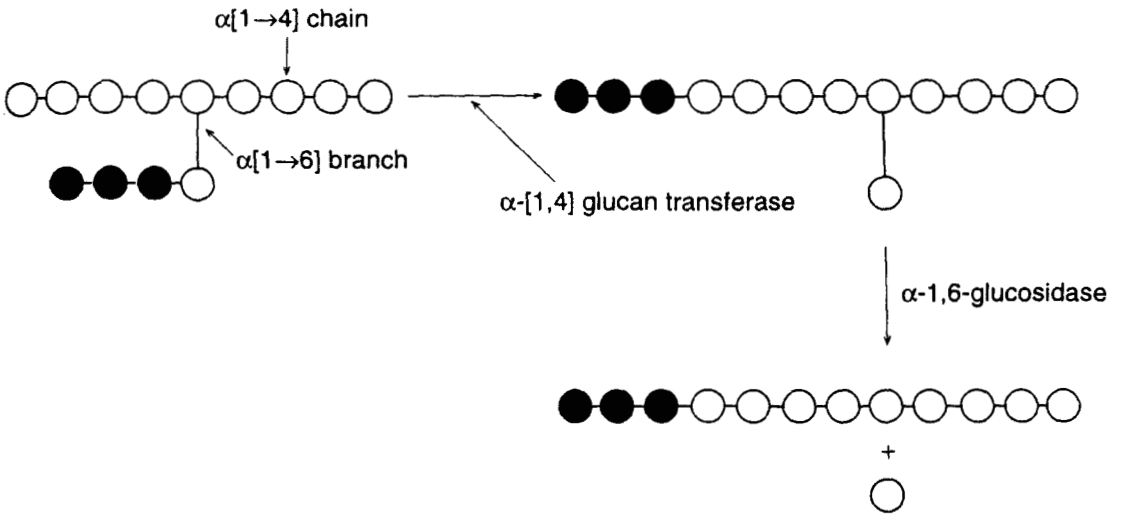


Figure 4-17. The glycogen debranching enzymes.

Glycogen phosphorylase will remove glucose units until it gets to within 4-5 glucose units of a branch point, when it no longer binds efficiently to the partially degraded glycogen.

2. **Debranching enzymes.** The complete degradation of glycogen requires two additional enzymes that make up the "debranching system" (Figure 4-17).
   a. **α-[1,4] → α-[1,4] glucan transferase** removes three or four glucose units from a branch point and transfers them to the end of another chain. In this reaction, one α-1,4 bond is being cleaved and another is being formed. The elongated chain now becomes a substrate for glycogen phosphorylase.
   b. **α-1,6 glucosidase** removes the single glucose unit remaining at the branch point and releases it as free glucose.

C. **Key enzymes in glycogen synthesis.** As shown in Figure 4-16, the synthesis of glycogen begins with glucose phosphorylation to glucose-6-phosphate. This reaction is catalyzed by glucokinase in the liver and by hexokinase in other tissues. Glucose-6-phosphate is rapidly and reversibly converted to glucose-1-phosphate by **phosphoglucomutase.** Before glucose can be added to the glycogen polymer, it must be "activated" or energized. This is achieved by the formation of **UDP-glucose,** which serves as the donor of glucose to a glycogen primer. Most monosaccharides are activated by reacting with UTP to form a UDP-sugar.

   1. **Formation of UDP-glucose.** The phosphate group of glucose-1-phosphate reacts with UTP to form UDP-glucose and pyrophosphate (PP_i). Thus, one of the phosphate groups in UDP-glucose is derived from glucose-1-phosphate and the other from UTP, as shown below. The reaction is catalyzed by

IN A NUTSHELL

Enzyme	*Activator*	*Inhibitor*
Muscle glycogen phosphorylase	*AMP* Ca^{2+} *Epinephrine*	*ATP Glucose-6-Phosphate Insulin*
Liver glycogen phosphorylase	*Glucagon Epinephrine*	*Glucose Insulin*
Glycogen synthase	*Glucose Insulin*	*Glucagon Epinephrine*

UDP-glucose pyrophosphorylase. The equilibrium of this reaction is "pulled" toward UDP-glucose formation by the hydrolysis of pyrophosphate to inorganic phosphate.

$$\text{Glu-1-P} + \text{Uridine-P-P-P} \xrightarrow{\text{UDP-glucose pyrophosphorylase}} \text{UDP-Glu} + PP_i \rightarrow 2\,P_i$$

The linkage between glucose and UDP is a high-energy bond, making it a suitable donor in many biosynthetic reactions.

2. **Elongation of glycogen chains.** To be active, glycogen synthase requires an existing glycogen chain serving as a primer. If the chain has been totally degraded, then glycogenin, the protein found at the core of the glycogen particle, serves as a primer for glycogen synthesis and does the initial synthesis. Glycogen synthase catalyzes the transfer of glucose from UDP-glucose to the end of a chain. The linkage created is an α-1,4-glycosidic bond. This is the rate-limiting step in glycogen synthesis and is therefore the site of regulation.

$$(\text{Glucose})_n + \text{UDP-glucose} \xrightarrow{\text{glycogen synthase}} (\text{Glucose})_{n+1} + \text{UDP}$$

3. **Branching enzyme.** After approximately 10 glucose units have been added, a branch point is created by α-[1,4] α-[1,6] glucan transferase. Forming a branch point involves breaking an α-1,4 linkage and creating an α-1,6 linkage. The two shorter chains that are produced can now be elongated by glycogen synthase. There are always at least four glucose molecules between branch points.

D. **Coordinate regulation of glycogenesis and glycogenolysis.** Glycogen phosphorylase and glycogen synthase are regulated in a reciprocal manner, such that when one of these enzymes is active, the other is inactive. The primary mode of regulation for both enzymes is hormonal and is mediated by phosphorylation/dephosphorylation. In addition, both glycogen synthase and phosphorylase activities can be affected allosterically.

1. **Glycogen phosphorylase.** A comprehensive diagram describing the regulation of this enzyme is shown in Figure 4-18. Phosphorylation of a specific serine side chain activates the enzyme, resulting in glycogen degradation. The activation of a phosphorylase is initiated by the binding of **glucagon** to liver cell receptors or by the binding of **epinephrine** to muscle receptors. Both of these hormones increase the intracellular

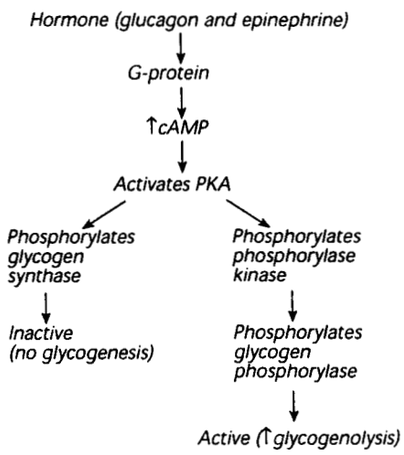
CLINICAL CORRELATE

Glycogenesis is coupled to the influx of K^+ in the cells. Insulin and glucose are therefore given to treat hyperkalemia (high serum K^+), inducing glycogenesis and causing an influx of K^+ into the cells.

synthesis of cAMP, resulting in the activation of a cAMP-dependent protein kinase (protein kinase A). A single molecule of hormone can generate many molecules of cAMP; each molecule of cAMP-dependent protein kinase can phosphorylate (and activate) many molecules of phosphorylase kinase; each molecule of phosphorylase kinase can phosphorylate (and activate) many molecules of glycogen phosphorylase; and, finally each molecule of glycogen phosphorylase can release many molecules of glucose-1-phosphate from glycogen. This "cascade" system amplifies the response initiated by the binding of a few hormone molecules by generating millions of molecules of glucose-1-phosphate. It is noteworthy that phosphorylase kinase can also be activated directly (without phosphorylation) by binding calcium. This is particularly important in skeletal muscle, where the coupling of excitation with contraction is mediated by calcium. Thus, the same regulatory molecule that activates contraction also activates glycogenolysis, which provides the energy for contraction. The active (phospho) form of glycogen phosphorylase can be allosterically inhibited by glucose and ATP, while the inactive (dephospho) form can be activated allosterically by AMP. Thus, although covalent modification of these enzymes is the primary mode of regulation, the activity of the phospho- and dephospho- forms can be fine-tuned by the accumulation of intracellular metabolites that act as allosteric effectors.

2. **Glycogen synthase.** The same signals that activate glycogen phosphorylase inactivate glycogen synthase. Glucagon and epinephrine (via cAMP-dependent protein kinase) stimulate the phosphorylation and inactivation of glycogen synthase. The coordinate regulation of these enzymes prevents futile cycling of substrate that would exist if both enzymes were active at the same time. The inactive (phosphorylated) form of glycogen synthase in muscle can be allosterically activated by glucose-6-phosphate. Many texts refer to glycogen synthase-I and glycogen synthase-D. The D form is the inactive (phospho) enzyme, while the I form is the active (dephospho) form.

IN A NUTSHELL



HEXOSE MONOPHOSPHATE (HMP) SHUNT

This pathway, also known as the **pentose phosphate pathway,** provides an alternative route for the oxidation of glucose. The products of the pathway are CO_2, pentose phosphates, and NADPH. The shunt branches off of glycolysis at glucose-6-phosphate, and re-enters at fructose-6-phosphate. The HMP shunt is present in the cytosol of all cells. Unlike glycolysis, this pathway neither consumes nor produces ATP.

A. **Functions.** This pathway supplies the cell with NADPH and pentoses.

1. **NADPH.** Almost all of the NADPH required in reductive biosynthetic processes, such as the synthesis of cholesterol, fatty acids, and steroid hormones, comes from the hexose monophosphate shunt. Tissues that synthesize large amounts of these compounds have high levels of the NADPH-producing enzymes in the pathway. Red blood cells require large amounts of NADPH to maintain the reduced form of glutathione. Reduced glutathione helps prevent hemolysis by neutralizing the effects of strong oxidizing agents such as superoxide and hydrogen peroxide. Neutrophils, macrophages, monocytes, and other phagocytosing cells require large quantities of NADPH to generate superoxide. These cells use superoxide as a part of the "cidial" process for killing bacteria they engulf. NADPH oxidase reduces molecular oxygen to superoxide in these cells. A deficiency in either NADPH or NADPH oxidase can result in chronic infection.

2. **Pentoses.** The synthesis of nucleotides and some coenzymes (NAD^+, $NADP^+$, FAD, CoA) require ribose-5-phosphate, which is supplied by the HMP shunt.

B. **Pathway and key enzymes.** As shown in Figure 4-19, the HMP shunt branches off glycolysis at glucose-6-phosphate. The two pathways equilibrate by having fructose-6-phosphate as a common intermediate. After the early steps in the pathway, which lead to NADPH and ribose-5-phosphate synthesis, a large number of intermediates (shown in brackets) allow excess pentoses to be reconverted to fructose-6-phosphate. The numbers at each reaction refer to the enzyme used. The reactions in the HMP shunt can be divided in two phases, the oxidative and nonoxidative phases.

1. **The oxidative phase** produces 2 mols of NADPH per glucose oxidized. This phase consists of three reactions starting with glucose-6-phosphate and resulting in **ribulose-5-phosphate.** All of these reactions are essentially irreversible. In the first reaction, the carbon-1 of glucose-6-phosphate is oxidized to

CLINICAL CORRELATE

Chronic granulomatous disease is caused by a defect in the NADPH oxidase complex, resulting in neutrophils that do not produce superoxide. Affected individuals therefore present with an inability to kill invading microbes that are engulfed.

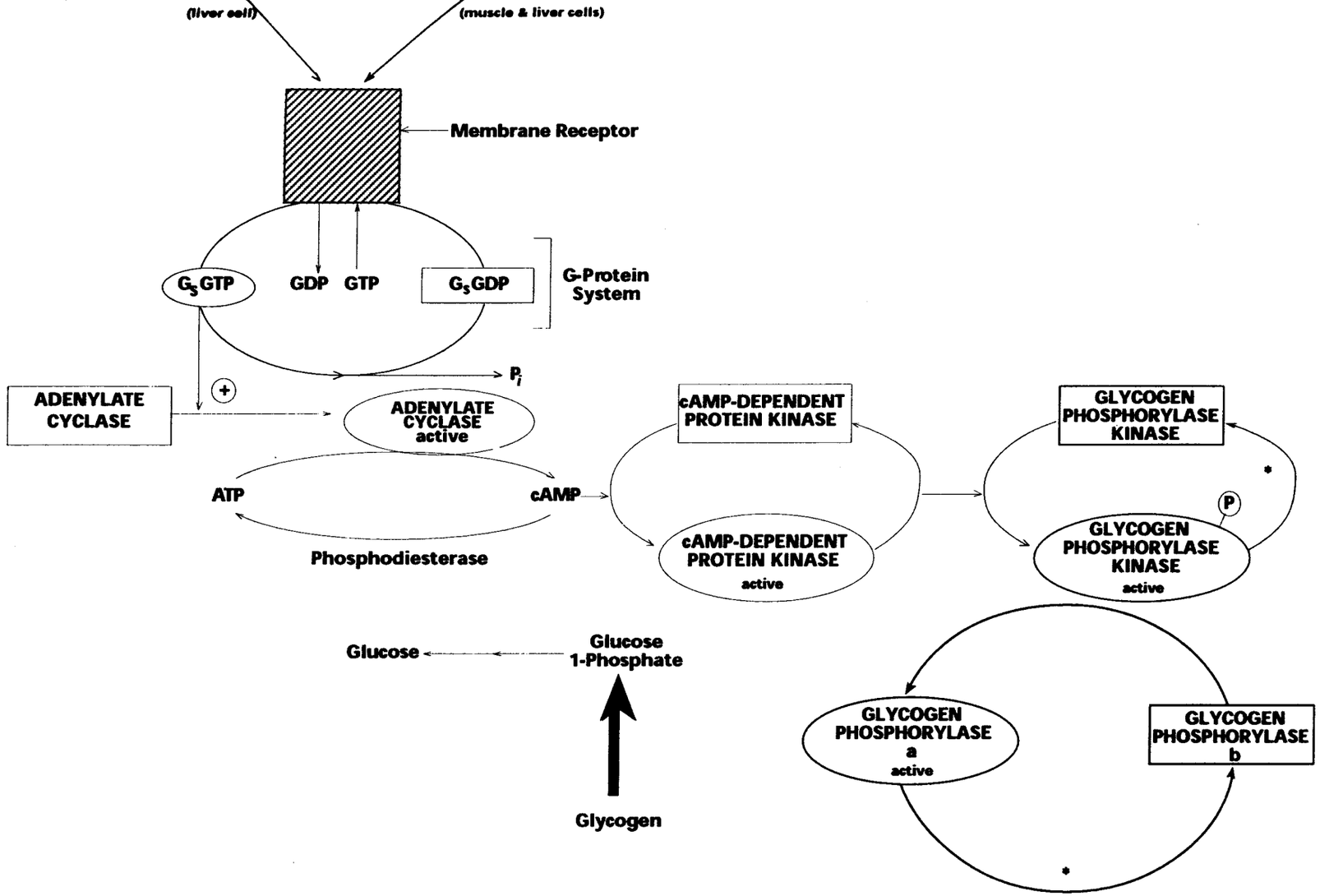


Figure 4-18. Cascade for activating glycogenolysis.

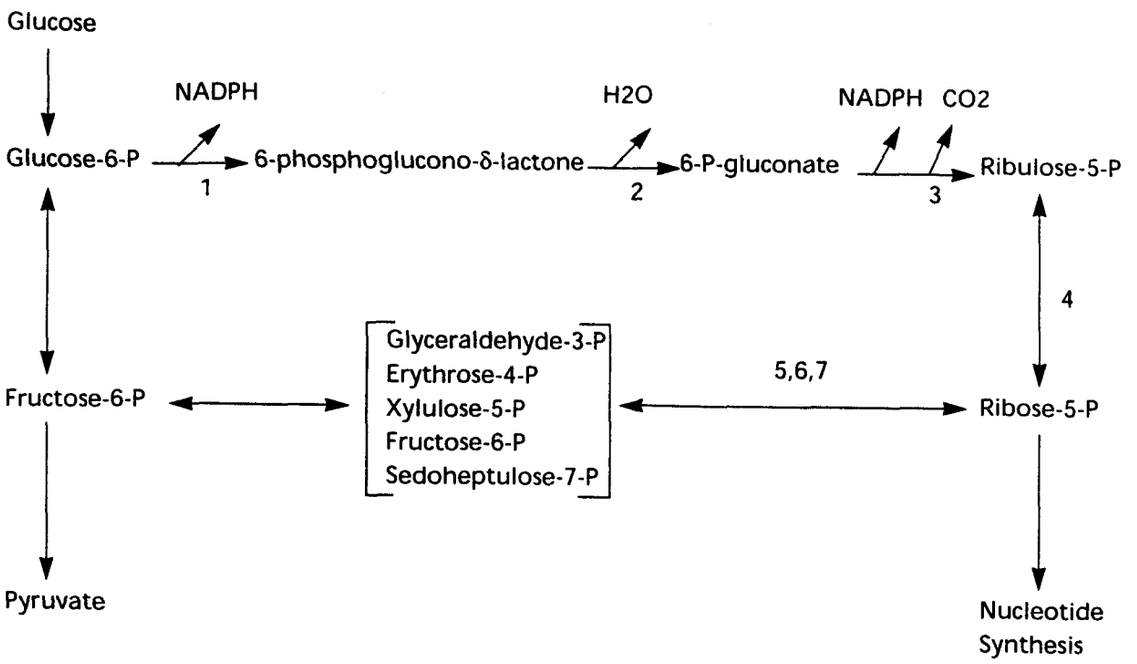


Figure 4-19. The hexose monophosphate shunt. 1. Glucose-6-phosphate dehydrogenase; 2. Lactonase; 3. 6-Phosphogluconate dehydrogenase; 4. Pentose-P-isomerase; 5. Pentose-P-epimerase; 6. Transketolase; 7. Transaldolase

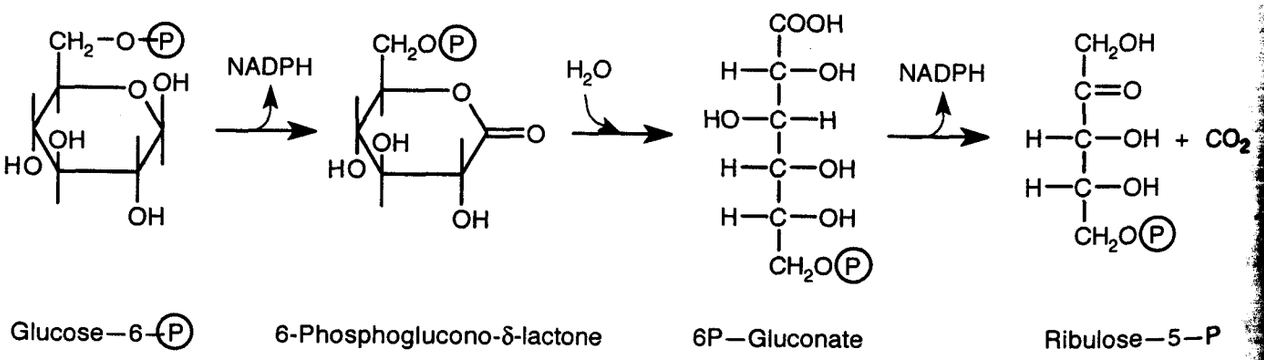


Figure 4-20. The oxidative phase of the hexose monophosphate shunt.

cyclic acid (lactone), with the simultaneous production of NADPH (Figure 4-20). In the next reaction, the lactone of 6-phosphogluconate is hydrolyzed to the straight chain form by lactonase. In the last step of the oxidative phase, 6-phosphogluconate is oxidized and decarboxylated, with the formation of NADPH, CO_2, and ribulose-5-phosphate.

2. **The nonoxidative phase.** All of these reactions are reversible. Ribulose-5-phosphate is isomerized to ribose-5-phosphate, th

pentose required for nucleotide synthesis. The remaining reactions involve the transfer of C_2- and C_3-units from one sugar to another. Intermediates having 3, 4, 5, 6, and 7 carbons are involved. The key enzymes in the transfer reactions are **transketolase** and **transaldolase.** Transketolase transfers C_2-units, using thiamine pyrophosphate as a C_2-carrier between donor and acceptor. Transaldolase transfers C_3 units. The major function of the nonoxidative phase is to provide a pathway for recycling excess pentoses. This is particularly important in tissues that require larger amounts of NADPH than pentoses. The recycling is achieved by the formation of fructose-6-phosphate, a common intermediate in the HMP shunt and glycolysis. Fructose-6-phosphate can be isomerized back to glucose-6-phosphate and reutilized. For every 6 mols of glucose-6-phosphate used in the HMP shunt, 6 mols of CO_2 are produced and 5 mols of fructose-6-phosphate can be returned to glycolysis.

C. **Regulation of the HMP shunt.** The rate-limiting step in the pathway is the initial reaction catalyzed by glucose-6-phosphate dehydrogenase. The amount of this enzyme present in liver and adipose increases when the diet contains large amounts of carbohydrate, a condition that leads to fatty acid synthesis and an increased requirement for NADPH. The activity of glucose-6-phosphate dehydrogenase is allosterically activated by NAD^+ and inhibited by NADH and palmitoyl-CoA.

FRUCTOSE METABOLISM

Fructose is second to glucose as a dietary source of carbohydrates. It is derived from the hydrolysis of sucrose in the brush border of the small intestine. The primary site of fructose metabolism is the liver. As the portal blood enters the liver, fructose is very efficiently removed and metabolized by the liver. The liver has three enzymes (fructokinase, aldolase B, and glyceraldehyde kinase) that convert fructose into trioses, intermediates in glycolysis. Since these reactions bypass the rate-limiting step in glycolysis, fructose is metabolized to pyruvate and acetyl-CoA much more rapidly than is glucose. The enzymes of glycolysis, gluconeogenesis, and glycogenesis also allow dietary fructose to be converted to blood glucose or glycogen. The very rapid phosphorylation of fructose by fructokinase may result in a transient decrease in intracellular phosphate concentrations and a diminished ability to synthesize ATP.

A. **Pathway and key enzymes of fructose metabolism.** The metabolism of fructose starts with the conversion to fructose-1-phosphate by fructokinase (Figure 4-21). Fructose-1-phosphate is split into two C_3 fragments by aldolase B, producing dihydroxyacetone phosphate and glyceraldehyde. Glyceraldehyde can be phosphorylated to glyceraldehyde-3-phosphate by triokinase. Alternatively, glyceraldehyde can be reduced to glycerol and used for gluconeogenesis or for triacylglycerol synthesis. Both dihydroxyacetone phosphate and glyceraldehyde-3-phosphate are intermediates in glycolysis. They can be further degraded by glycolysis or they be condensed to fructose-1,6-bisphosphate and used for gluconeogenesis or glycogenesis.

B. **Abnormalities in fructose metabolism** arise from deficiencies in **fructokinase** and **aldolase B,** enzymes unique to fructose catabolism. A deficiency in fructokinase results in essential fructosuria, a benign condition in which fructose appears in the blood and urine and is excreted intact. A deficiency in aldolase B results in hereditary fructose tolerance.

CLINICAL CORRELATE

Hereditary fructose intolerance (HFI) results from a deficiency of aldolase B, the enzyme that cleaves fructose-1-phosphate to glyceraldehyde and dihydroxyacetone. This results in an accumulation of fructose-1-phosphate in the liver, which inhibits glycogen phosphorylase and aldolase. Glycogenolysis and gluconeogenesis are thereby both impaired, resulting in severe hypoglycemia and vomiting. Symptoms are reversed after removing fructose and sucrose from the diet.

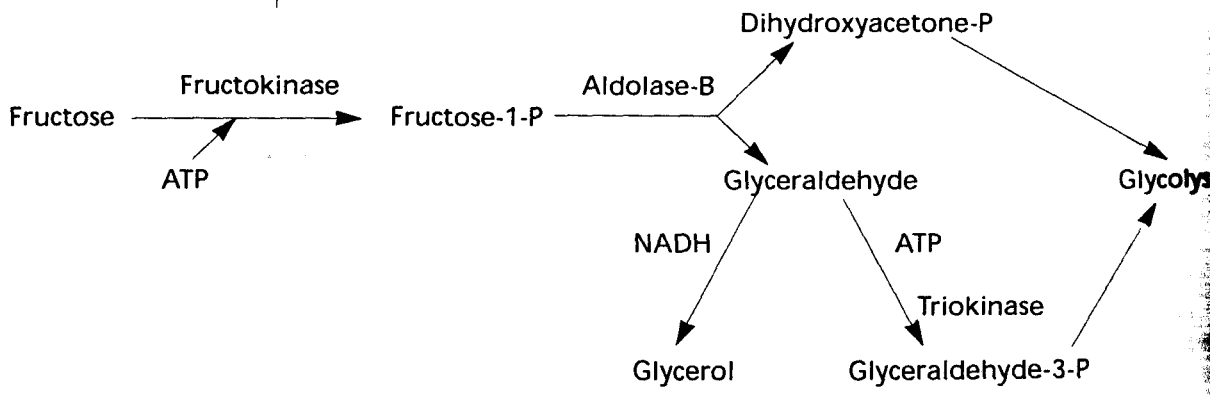


Figure 4-21. Fructose metabolism by the liver.

GALACTOSE METABOLISM

The primary source of galactose is milk, which contains high amounts of lactose. The concentration of lactose is higher in human milk than in cow's milk, although the caloric intake is about the same. Lactose is hydrolyzed in the small intestine to glucose and galactose. The metabolism of galactose occurs almost entirely in the liver. Three enzymes (galactokinase, gal-1-P:glu-1-P uridyl transferase, and UDP-galactose-4-epimerase) are required to assimilate galactose into the central pathways of carbohydrate metabolism.

CLINICAL CORRELATE

Galactosemia is an autosomal recessive disease resulting from a deficiency in either galactokinase or galactose-1-phosphate uridyl transferase. In both deficiencies, galactose accumulates and is converted to galactitol in the nervous system (retardation) and lens of the eye (cataracts). In the uridyl transferase deficiency, galactose-1-phosphate accumulates in the liver, resulting in hepatomegaly. Treatment requires excluding galactose (and lactose) from the diet.

A. **Pathway and key enzymes of galactose metabolism.** Galactose metabolism starts by phosphorylation to galactose-1-phosphate by **galactokinase.** All dietary galactose is phosphorylated in the liver (Figure 4-22). Galactose-1-phosphate is then exchanged for the glucose-1-phosphate moiety of UDP-glucose. This reaction is catalyzed by "**uridyl transferase**" and results in glucose-1-phosphate and UDP-galactose. Finally, UDP-galactose is recycled to UDP-glucose by UDP-galactose-4-epimerase.

The overall effect of these three reactions is to convert dietary galactose into glucose-1-P. Through pathways already considered in this chapter, glucose-1-phosphate can be incorporated into glycogen or converted to glucose-6-phosphate. The glucose-6-phosphate can be hydrolyzed by glucose-6-phosphatase to release glucose into the blood, or it can be used by glycolysis to produce pyruvate.

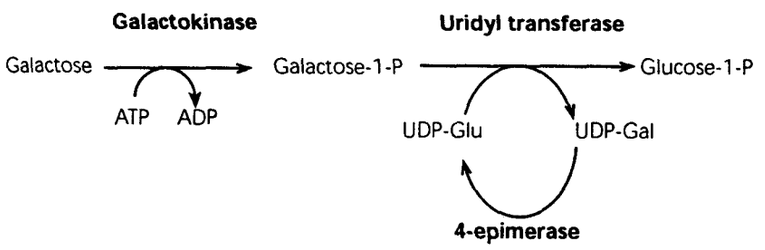


Figure 4-22. Metabolism of galactose by liver.

ipids

Lipids are biologically important compounds that are structurally and functionally diverse. They can be classified into six major groups: fatty acids, triacylglycerols, ketone bodies, cholesterol, phospholipids, and sphingolipids. Each class of lipids plays a specific role in either energy production, transport, and storage, or as a structural component of cell membranes. Numerous diseases including obesity, diabetes, hyperlipoproteinemias, and sphingolipidoses are characterized by abnormalities in lipid transport and/or metabolism. Other minor classes of lipids, including bile acids, fat-soluble vitamins, and eicosanoids play important roles in normal body function as well as in certain disease states. This chapter describes the major classes of lipids, their cellular functions, and the metabolic pathways that connect them.

CLASSIFICATION OF LIPIDS

The major classes of lipids and their functions are listed in Table 5-1. A brief description of the major structural features of each class follows the table.

LIPID	FUNCTION
Fatty acid	Metabolic fuel; building block for triacylglycerol, phospholipids, and sphingolipids
Triacylglycerols	Storage depot and major transport form for fatty acids
Ketone bodies	Soluble metabolic fuel for skeletal muscle, cardiac muscle, kidney, and brain
Cholesterol	Structural component of plasma membrane; precursor of bile acids, vitamin D, and steroid hormones
Phospholipids	Major building block of membranes; storage site for polyunsaturated fatty acids; signal transduction pathways
Sphingolipids	Structural component of membranes; surface antigens

Table 5-1. Major classes of lipids and their metabolic functions.

A. **Fatty acids** are composed of a long hydrocarbon chain with a carboxyl group at one end. They are amphipathic molecules, containing both polar and nonpolar ends. Fatty acids can be described using two different numbering systems. The C-numbering system starts at the carboxyl end and the ω-numbering system starts at the methyl end. The α-carbon is adjacent to the carboxyl group. Fatty acids are frequently identified as either "saturated" or "unsaturated" depending upon the absence or presence of double bonds, respectively.

1. **Saturated fatty acids** contain no double bonds. The most common saturated fatty acids are palmitic and stearic acid. Their formulae and those of other fatty acids found in human tissues are listed Table 5-2.

Fatty acid	Length	Structure
Acetic	C_2	CH_3-COOH
Propionic	C_3	CH_3-CH-COOH
Butyric	C_4	CH_3-$(CH_2)_2$-COOH
Lauric	C_{12}	CH_3-$(CH_2)_{10}$-COOH
Myristic	C_{14}	CH_3-$(CH_2)_{12}$-COOH
Palmitic	C_{16}	CH_3-$(CH_2)_{14}$-COOH
Stearic	C_{18}	CH_3-$(CH_2)_{16}$-COOH
Arachidic	C_{20}	CH_3-$(CH_2)_{18}$-COOH
Lignoceric	C_{24}	CH_3-$(CH_2)_{22}$-COOH

Table 5-2. Length and structure of common saturated fatty acids.

2. **Unsaturated fatty acids** contain one or more double bonds in the cis-configuration.
   a. **Monounsaturated fatty acids** contain one double bond. The most common monounsaturated fatty acid is the 18-carbon oleic acid, which has one double bond between carbons 9 and 10: CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_7$-COOH.
   b. **Polyunsaturated fatty acids** contain two or more double bonds.
      (1) **Linoleic acid** is an 18-carbon ω-6 fatty acid with two double bonds. It is an essential fatty acid because it must be obtained from dietary sources.

$$CH_3\text{-}(CH_2)_4\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COOH$$

      (2) **Linolenic acid** is an 18-carbon ω-3 essential fatty acid with three double bonds.

$$CH_3\text{-}CH_2\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COOH$$

CLINICAL CORRELATE

A deficiency of essential fatty acids (linoleic and linolenic acids) is seen in patients on long-term IV hyperalimentation with no IV fat supplementation.

(3) **Arachidonic acid** is a 20-carbon ω-6 fatty acid with four double bonds. It can be synthesized by humans from linoleic acid via elongation and desaturation reactions. If linoleic acid is deficient in the diet, arachidonic acid becomes an essential fatty acid.

. **Triacylglycerols (TAG)** contain a glycerol backbone with three fatty acids linked as esters. This is the most common storage form for fatty acids.

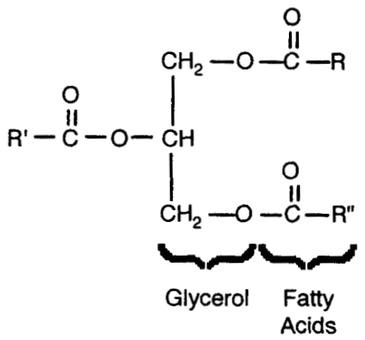


Figure 5-1. Triacylglycerol.

Removal of one fatty acid generates a **diacylglycerol,** which acts as a second messenger in the phosphatidylinositol signal transduction pathway. Removal of fatty acids from both ends of the triacylglycerol molecule results in 2-monoglyceride, the end product of TAG digestion in the small intestine.

. **Ketone bodies** are C_4 acids that have either a keto or a hydroxyl group attached to the β-carbon atom. The two major ketone bodies are **acetoacetic acid** and **β-hydroxybutyric acid** (Figures 5-2 and 5-3).

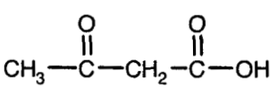


Figure 5-2. Acetoacetic acid.

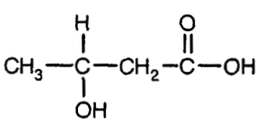


Figure 5-3. β-Hydroxybutyric acid.

). **Cholesterol and sterol derivatives** contain a common steroid nucleus—a fused four-member ring system that contains 19 carbon atoms (Figure 5-4). Cholesterol, a 27-carbon compound

AN

CLINICAL CORRELATE

Arachidonic acid is the major precursor for prostaglandins, thromboxanes, and leukotrienes, which act as local hormones and play critical regulatory roles in human physiology. The biological action of these compounds is different for each organ system. Platelet aggregation is controlled by the antagonistic effects of prostaglandins and thromboxanes; leukotrienes are important mediators of inflammation and allergic responses. A deficiency in arachidonic acid and an overproduction of eicosanoids from arachidonic acid both result in a diverse group of symptoms.

CLINICAL CORRELATE

Ketone bodies are synthesized from fatty acids and amino acids in the liver during prolonged starvation and diabetic ketoacidosis. They are excreted in the urine, but β-hydroxybutyric acid will not show up on a urine ketone test — the brain metabolizes it to two acetyl CoA molecules. Patients with excess ketones present with a fruity breath odor.

(Figure 5-5), is the precursor for vitamin D (C_{27}), bile acids (C_{24}), adrenocortical hormones (C_{21}), progesterone (C_{21}), androgens (C_{19}), and estrogens (C_{18}).

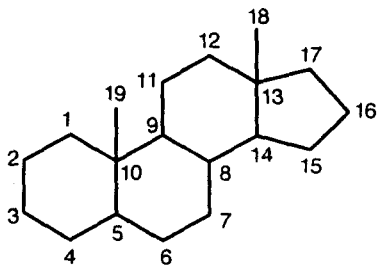


Figure 5-4. Steroid nucleus.

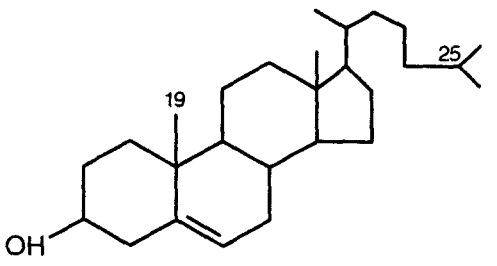


Figure 5-5. Cholesterol.

E. **Phospholipids** are amphipathic molecules consisting of two alcohols linked by a phosphodiester bridge. **Diacylglycerol**, the alcohol common to all of the phospholipids, contains the nonpolar structural component. The polar head is contributed by the second alcohol. It is this polar head that distinguishes each class of phospholipids. Choline, ethanolamine, and serine are amino alcohols, whereas inositol is a polyol. The four most important glycerol-based phospholipids can all be represented by the structure shown in Figure 5-6, where the nature of X is unique for each phospholipid. The names of these phospholipids, the nature of X, and its structure are given in Table 5-3.

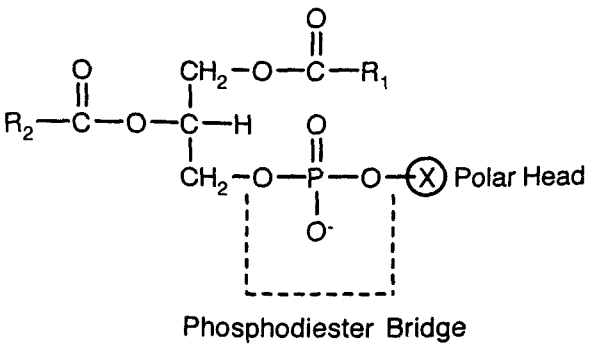


Figure 5-6. Glycerol-based phospholipid.

CLINICAL CORRELATE

Respiratory distress syndrome (RDS) occurs in preterm newborns due to the inability of the premature lung to synthesize dipalmitoylphosphatidylcholine, the active component of pulmonary surfactant. This lipid, which is normally synthesized shortly prior to parturition, forms a film that lines the alveoli and reduces the surface tension of the aqueous layer, thereby preventing the alveoli from collapsing during exhalation. Symptoms at birth include hypoxia, acidosis, and pulmonary edema. The maturity of the fetal lung can be assessed prenatally by measuring the ratio of dipalmitoylphosphatidylcholine/sphingomyelin in amniotic fluid. Glucocorticoids (dexamethasone) can be used to induce production of dipalmitoylphosphatidylcholine and develop the lungs more quickly.

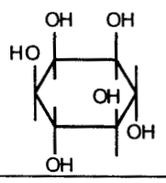
hospholipid	X	Structure
hosphatidylethanolamine (cephalin)	Ethanolamine	$HO-CH_2-CH_2-\overset{+}{N}H_3$
hosphatidylcholine (lecithin)	Choline	$HO-CH_2-CH_2-\overset{+}{N}(CH_3)_3$
hosphatidylserine	Serine	$HO-CH_2-CH(COOH)-\overset{+}{N}H_3$
hosphatidylinositol	Inositol	

Table 5-3. The unique polar heads of selected glycerol-based phospholipids.

In membranes, the polar head group faces the aqueous environment and the fatty acid groups of diacylglycerol constitute the lipid bilayer.

Sphingolipids and **glycolipids** contain **ceramide** as a common structural component. Ceramide is composed of **sphingosine**, a long-chain amino alcohol with a saturated fatty acid linked to the amino group (Figure 5-7). The classes of sphingolipids and glycolipids can be differentiated on the basis of the X-group that is esterified to the terminal hydroxyl group of ceramide (Table 5-4).

Sphingosine

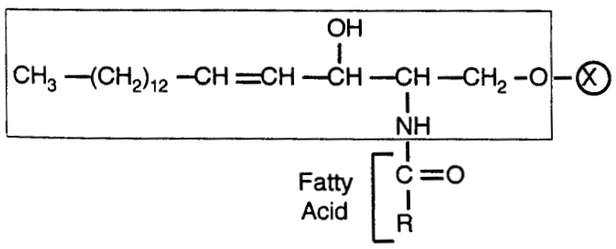


Figure 5-7. Sphingolipids and glycolipids.

Sphingolipid or glycolipid	X	Location
Sphingomyelin	Phosphocholine	Myelin sheath
Cerebrosides	Single hexose	Brain, peripheral nerve
Sulfatide	Galactose-SO_4	Nerve tissue
Globoside	Oligosaccharide	RBC membrane
Ganglioside	Oligosaccharide with N-acetylneuraminic acid	Ganglion cells

Table 5-4. The unique X-groups of selected sphingolipids and glycolipids.

LIPOPROTEINS

The metabolism of lipids frequently requires that a particular lipid be transported in the blood between different organs. The main vehicles for transporting neutral lipids in the blood are the lipoproteins.

A. **Structure and composition.** All lipoproteins consist of a hydrophilic shell and a hydrophobic core. The hydrophilic shell contains proteins, phospholipids, and unesterified cholesterol—amphipathic molecules that interact favorably with the aqueous environment. The hydrophobic core contains the neutral lipids, triacylglycerols, and cholesterol esters, which are highly insoluble in water. A model for the structure of lipoproteins is shown in Figure 5-8.

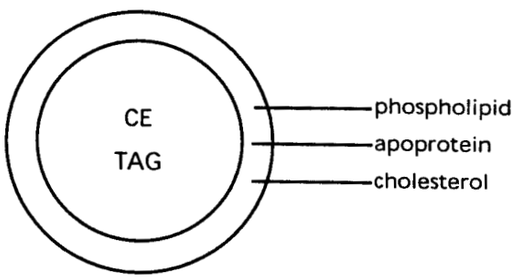


Figure 5-8. Lipoprotein structure.

B. **Classes.** The four major classes of lipoproteins in human serum can be separated by electrophoresis on the basis of their size and charge or by centrifugation on the basis of their density. A typical electrophoretogram is shown in Figure 5-9 and a typical centrifugal separation is shown in Figure 5-10.

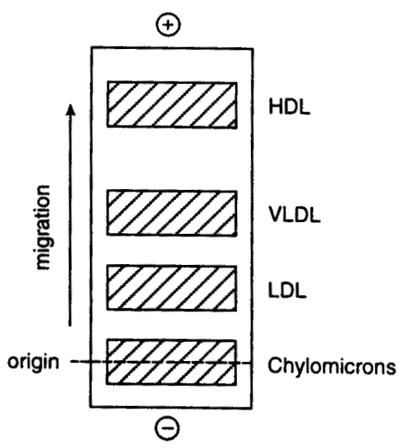


Figure 5-9. Lipoprotein electrophoresis.

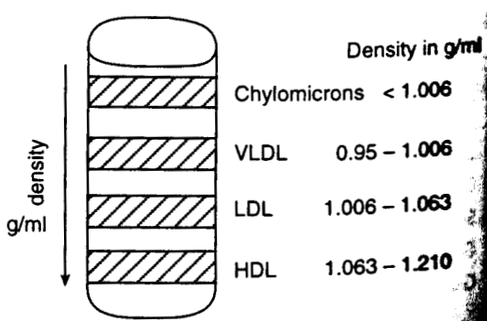


Figure 5-10. Lioprotein centrifugation.

1. **Chylomicrons** are the least dense of the lipoproteins and do not migrate in an electric field. They are formed in intestinal mucosa and transport dietary triacylglycerol (TAG) and cholesterol ester (CE). Chylomicrons are synthesized in the smooth ER of intestinal epithelial cells. The TAGs in chylomicrons are hydrolyzed by **lipoprotein lipase,** an enzyme attached to the luminal surface of the vasculature of cardiac muscle, skeletal muscle, and adipose tissue. Chylomicrons contain several apoproteins, including apo B_{48}, apo E, and apo C-II. Apo B_{48} is unique to chylomicrons; apo C-II activates lipoprotein lipase, resulting in fatty acid release to heart, skeletal muscle, and mammary glands. The presence of apo E facilitates the clearance of chylomicron remnants by the liver.

CLINICAL CORRELATE

A deficiency in lipoprotien lipase will result in hyperchylomicronemia, or Type I hyperlipoproteinemia.

2. **Very low-density lipoproteins (VLDLs)** are synthesized in the liver and transport TAG and CE. VLDLs are composed mainly of TAG, yet are more enriched in CE than are chylomicrons. VLDLs contain apo B_{100}, apo E, and apo C-II. Like chylomicrons, VLDLs are metabolized by lipoprotein lipase to produce remnants (intermediate-density lipoproteins, IDLs). These remnants be can be further metabolized to particles of still lower density, or they can be internalized by the liver.
3. **Low-density lipoproteins (LDLs)** are generated from VLDLs and IDLs by the action of lipoprotein lipase, thus increasing the relative proportion of cholesterol esters in the neutral core. The major function of LDL is to transport cholesterol to extrahepatic tissues, where it is taken up by receptor-mediated endocytosis. The LDL particle retains only apo B, and the uptake of LDL by cells is initiated by the interaction of apo B with LDL receptors on the plasma membranes.

CLINICAL CORRELATE

Familial hypercholesterolemia (FH), or Type II hyperlipoproteinemia, arises as a result of defective LDL receptors and is characterized by elevated LDL cholesterol. A positive correlation exists between elevated plasma concentration of LDL and coronary atherosclerosis.

4. **High-density lipoproteins (HDLs)** are synthesized by the liver and are approximately 50% protein. When the particle is secreted by the liver, the core region is relatively empty. HDLs perform two major functions:
   a. **Circulating reservoir for apoproteins.** Members of the apo A, apo C, and apo E families can be transferred back and forth between other lipoproteins. Newly synthesized chylomicrons and VLDL particles obtain some of their apoproteins from the HDL reservoir following secretion.
   b. **Reverse cholesterol transport.** HDLs are important in moving cholesterol from extrahepatic tissues to the liver. Elevated plasma levels of HDL are associated with decreased incidence of coronary atherosclerosis. Cholesterol is taken up from the surface of cells by HDL, esterified to cholesterol esters, and ultimately returned to the liver either by uptake of HDL particles by the liver or by the transfer of cholesterol

esters to VLDL and chylomicron remnants followed by remnant uptake. Two proteins play important roles in reverse cholesterol transport:

(1) **Lecithin cholesterol acyl transferase (LCAT)** is a plasma enzyme that esterifies HDL cholesterol. The fatty acid used for esterification comes from lecithin (phosphatidylcholine). LCAT is activated by apo A-I, which is associated with HDL.

(2) **Cholesterol ester transfer protein (apo D)** is associated with HDL and facilitates the transfer of cholesterol esters to VLDL and chylomicron remnants in exchange for triacylglycerol.

C. **Abnormalities of lipoprotein metabolism** may result in either elevated or decreased plasma concentrations of certain lipoproteins and associated lipids.

FATTY ACID METABOLISM

There are six major pathways of fatty acid and lipid metabolism. Fatty acids are synthesized from excess carbohydrate in liver and adipose tissue. They are stored as triacylglycerols in adipose tissue when nutrition is sufficient, and are mobilized from triacylglycerol and released into the blood during fasting or exercise. Most of the free fatty acids that are released from adipose tissue are carried by serum albumin to skeletal muscle, cardiac muscle, and liver, where they undergo β-oxidization to provide energy to the tissues. Cholesterol and ketones are synthesized from acetyl-CoA. Ketones are utilized as a metabolic fuel, and fatty acids are used to synthesize specialized products, such as phospholipids, sphingolipids, glycolipids, and prostaglandins. An overview of these pathways is outlined in Figure 5-11.

The pathways in Figure 5-11 that predominate at any time are determined largely by the insulin/glucagon ratio. An increase in the insulin/glucagon ratio (due to insulin secretion when blood glucose is high) favors lipid synthesis and storage, whereas a decrease in the insulin/glucagon ratio (due to glucagon secretion when blood glucose is low) favors fatty acid mobilization and β-oxidation to generate energy. Many of these effects are mediated by the antagonistic effects of glucagon and insulin on the intracellular concentration of cAMP (glucagon increases cAMP and insulin decreases cAMP). Under conditions where excessive fatty acid oxidation is occurring and acetyl-CoA accumulates, ketone synthesis occurs as a means of converting acetyl-CoA into a soluble fuel that can be readily transported in the plasma. The key steps of these pathways, as well as the mechanism by which the pathways are regulated and coordinated, will be discussed separately.

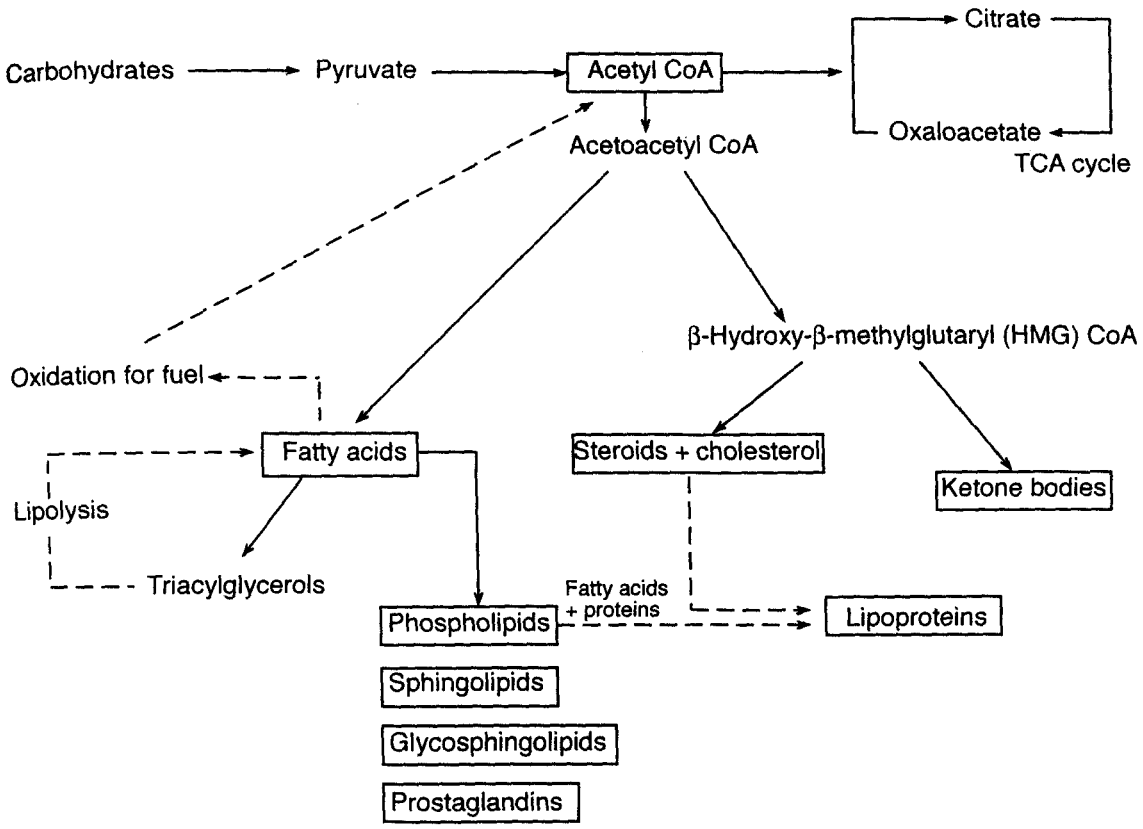


Figure 5-11. Inter-relationship of pathways of fatty acid metabolism.

A. **Fatty acid synthesis.** In humans, fatty acids are synthesized from acetyl-CoA, bicarbonate, and NADPH. The reactions that occur are essentially a reversal of β-oxidation, with two distinctions: the two processes use different enzymes and occur in different cellular compartments. Fatty acid synthesis occurs in the cytosol, whereas β-oxidation occurs in mitochondria. Humans can synthesize all of the required fatty acids except **linoleic acid** and **linolenic acid,** which are therefore essential dietary requirements. Before fatty acid synthesis can begin, acetyl-CoA must be translocated from the mitochondria, where it is produced, to the cytosol, where it is utilized. Since the inner mitochondrial membrane is impermeable to acetyl-CoA, the **citrate shuttle** is used to carry acetyl-CoA from the mitochondrial matrix to the cytosol. The key steps of the citrate shuttle are shown in Figure 5-12.

Citrate is formed in the mitochondria from oxaloacetate and acetyl-CoA and then transported into the cytosol. Once in the cytosol, citrate is cleaved by **citrate lyase** to form acetyl-CoA and oxaloacetate, which is converted through two sequential reactions to pyruvate. Pyruvate is returned to the mitochondria, where it is carboxylated to oxaloacetate to complete the shuttle. The citrate shuttle also produces **NADPH**, another factor required

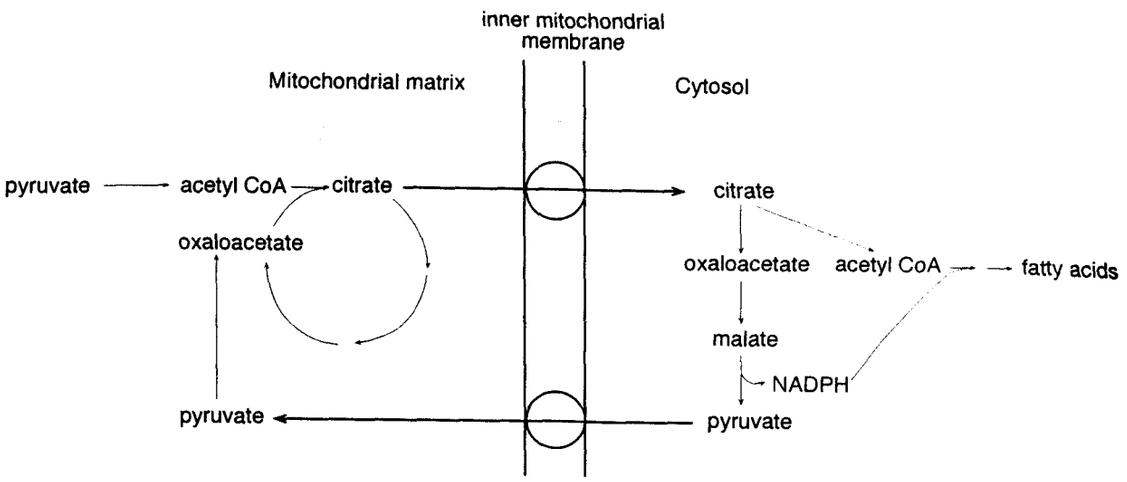


Figure 5-12. The citrate shuttle.

for fatty acid synthesis. About half of the NADPH for fatty acid synthesis is supplied by the pentose phosphate pathway.

1. **Key enzymes in fatty acid synthesis**
   a. **Acetyl-CoA carboxylase** catalyzes the first step in fatty acid synthesis. The enzyme requires the presence of both biotin and bicarbonate for converting acetyl-CoA to **malonyl-CoA** (Figure 5-13). Malonyl-CoA is a three-carbon compound that serves as the donor of two-carbon units in the elongation of the fatty acid chain.

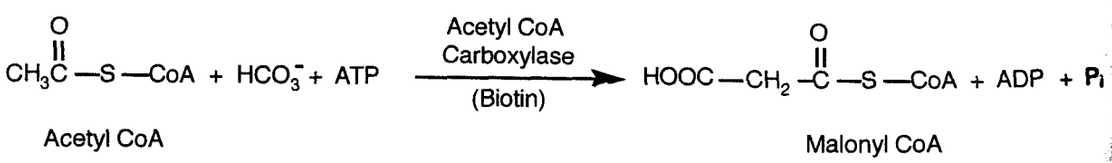


Figure 5-13. The conversion of acetyl-CoA to malonyl-CoA.

   b. **Fatty acid synthase (FAS)** is a multienzyme complex that catalyzes all of the remaining reactions. It lengthens the fatty acyl-CoA being synthesized by the sequential addition of two-carbon units to the carboxyl end. After each addition, it reduces the product to a fully saturated acyl group. All of the reactions occur while the intermediates are covalently bound as thioesters to either acyl-carrier protein (ACP) or to the condensing enzyme (synthase). Each cycle adds a two-carbon unit and uses two molecules of NADPH to reduce the ketoacyl intermediate to a fully saturated fatty acid. Each cycle consists of the four sequential reactions shown in Figure 5-14.

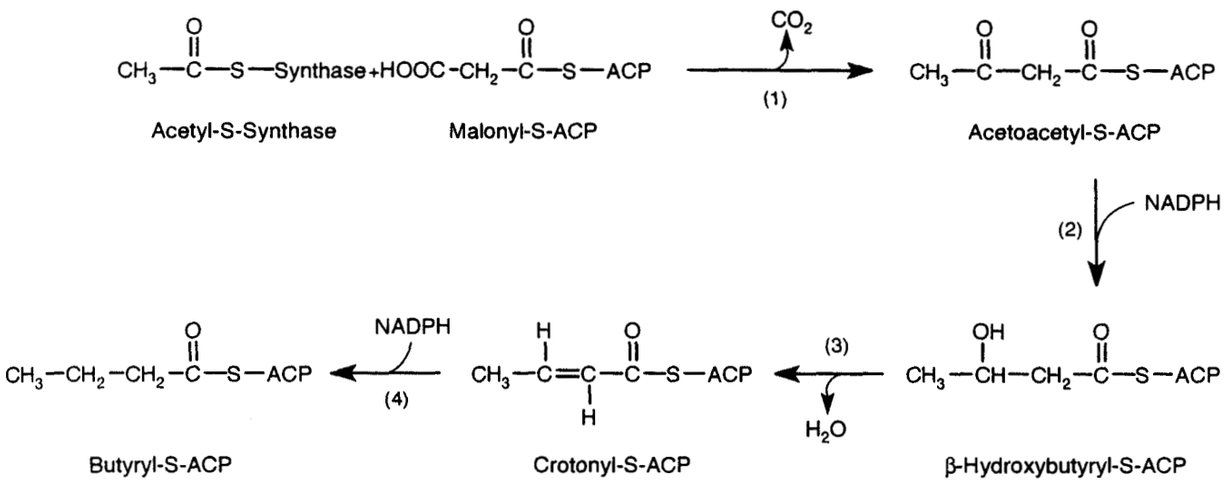


Figure 5-14. The enzymatic actions of FAS.

At the end of the first cycle, butyryl-CoA is transferred onto the synthase so that the acyl carrier protein can be recharged with another molecule of malonyl-CoA and a new cycle can begin. Each cycle consists of the following four reactions:

(1) **Condensation** of malonyl-CoA with fatty acyl-CoA to produce a β-ketoacyl-CoA intermediate.
(2) **Reduction** of β-ketoacyl-CoA to β-hydroxyacyl-CoA in an NADPH-dependent reaction.
(3) **Dehydration** of β-hydroxyacyl-CoA to produce enoyl-CoA with a double bond.
(4) **Reduction** of the double bond by NADPH to produce a fatty acyl-CoA that has been lengthened by two carbons. This cyclic pattern is repeated until **palmitoyl-CoA**, the product of the FAS complex, is formed.

2. **Elongation and desaturation** reactions convert palmitate into a variety of fatty acids that are required for use by the normal cell. These reactions, which occur primarily in the endoplasmic reticulum, rely upon the use of malonyl-CoA for elongation. Elongation can also occur in the mitochondria, where acetyl-CoA, instead of malonyl-CoA, is used for elongation. Desaturation occurs in the endoplasmic reticulum and requires a multienzyme complex that contains **cyt b_5 and cyt b_5 reductase**.

3. **Regulation of fatty acid synthesis** depends on the availability of substrates and the activity of acetyl-CoA carboxylase, the enzyme that catalyzes the first step in the initial reaction converting acetyl-CoA to malonyl-CoA. This reaction is the rate-limiting step in fatty acid synthesis. **Acetyl-CoA carboxylase** is

IN A NUTSHELL

Fatty acid synthesis:

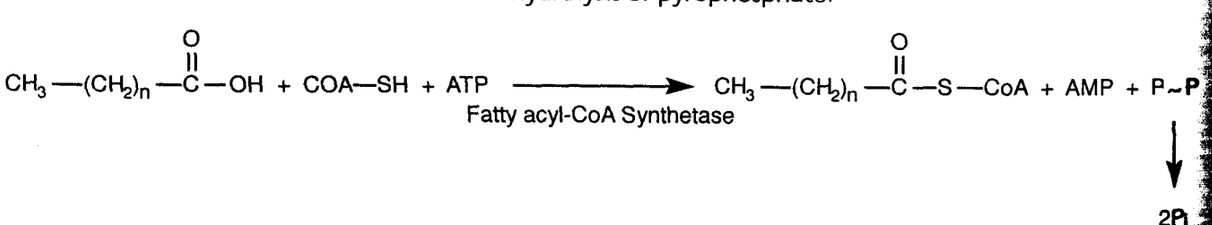
- *Occurs in cytosol*
- *Citrate shuttle (acetyl-CoA transport to cytoplasm, NADPH production)*
- *Acetyl-CoA carboxylase*
  - *first step/rate-limiting step*
  - *produces malonyl-CoA (C_2 donor)*
  - *uses 1 ATP*
- *Fatty acid synthase*
  - *lengthens fatty acid chain by two carbons from carboxy terminus*
  - *reduces ketone to full saturation*
  - *uses 2 NADPH*
- *Synthesis stops at C_{16} palmitoyl-CoA*
  - *needs microsomes from endoplasmic reticulum to elongate past C_{16}*
- *Synthesis of palmitoyl-CoA requires 7 ATP and 14 NADPH*

activated by citrate, insulin, and a high-carbohydrate, low-fat diet; it is inhibited by fatty acids, glucagon, and a high-fat diet. Acetyl-CoA carboxylase is inactivated by cAMP-dependent phosphorylation and activated by insulin-dependent dephosphorylation. The synthesis of both acetyl-CoA carboxylase and fatty acid synthase complex is induced by insulin.

B. **Fatty acid oxidation.** Most fatty acid oxidation occurs in the mitochondria, although peroxisomes are important in the oxidation of very long-chain fatty acids and branched-chain fatty acids. A separate set of enzymes oxidizes fatty acids in peroxisomes.

1. **Uptake and oxidation of fatty acids.** The uptake and oxidation of fatty acids by cells can be divided into three major stages: activation, the transfer of fatty acids into the mitochondria, and β-oxidation of fatty acids.

a. **Activation.** Fatty acids are converted to fatty acyl-CoA immediately upon entry into the cell. This reaction traps the fatty acid in the cell and generates a high-energy thioester bond with CoA-SH. The reaction is catalyzed by **fatty acyl-CoA synthetase (thiolase)** and is driven by the hydrolysis of pyrophosphate.



b. **Transfer of fatty acids into mitochondria.** Fatty acids enter the mitochondria via the **carnitine shuttle,** which consists of two enzymes (i.e., carnitine-acyl transferase-I and carnitine-acyl transferase-II) and one transporter (i.e., carnitine translocase).

(1) **Carnitine-acyl transferase-I (CAT-I)** is associated with the outer surface of the inner mitochondrial membrane. It transfers the fatty acid from fatty acyl-CoA to carnitine to form fatty acyl carnitine.

(2) **Carnitine translocase** transports fatty acyl carnitine into the mitochondria and transports free carnitine back out of the mitochondria.

(3) **Carnitine-acyl transferase-II (CAT-II)** is associated with the inner surface of the inner mitochondrial membrane. It catalyzes the reformation of fatty acyl-CoA in the mitochondrial matrix.

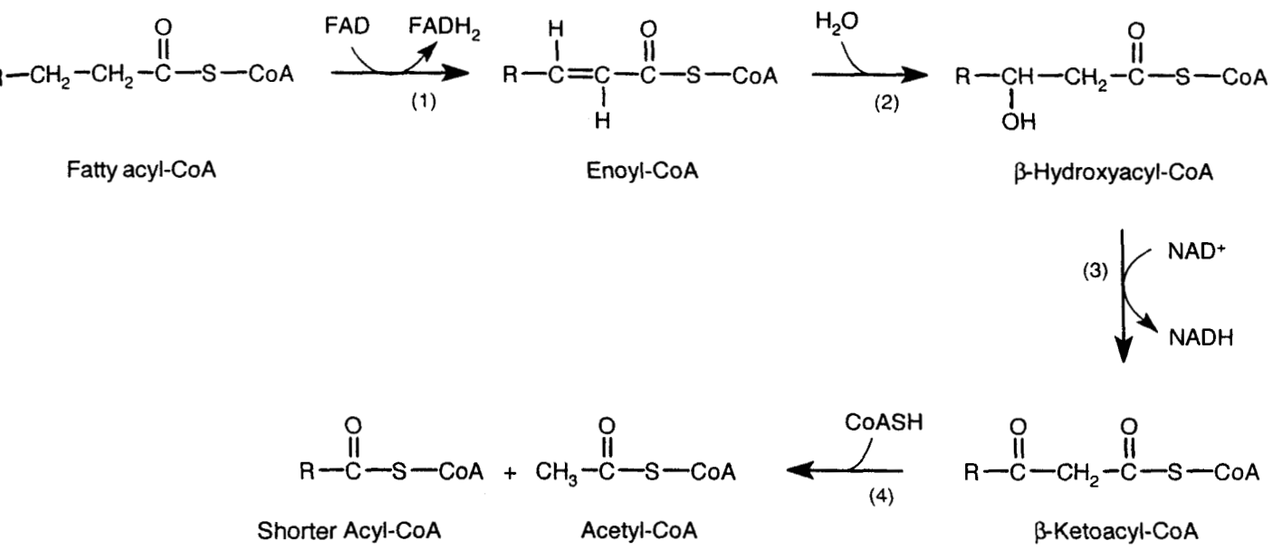


Figure 5-15. β-oxidation of fatty acids.

c. **β-oxidation of fatty acids** involves the sequential removal of two-carbon fragments from the carboxyl end. Each cycle of oxidation involves four reactions and generates one molecule each of acetyl-CoA, NADH, and $FADH_2$. The pathway is called β-oxidation because the two oxidation steps that generate $FADH_2$ and NADH both involve the β-carbon atom. The key reactions are shown in Figure 5-15.
   (1) **Dehydrogenation** of the fatty acid forms a double bond between the α and β carbons in a reaction that generates $FADH_2$. This reaction is catalyzed by a family of fatty acyl-CoA dehydrogenases that differ in specificity for fatty acid chain length.
   (2) **Hydration** of the double bond produces β-hydroxy-acyl-CoA.
   (3) **Dehydrogenation** of β-hydroxyacyl-CoA in an NAD^+-dependent reaction generates β-ketoacyl-CoA.
   (4) **Thiolytic cleavage** by the addition of CoA-SH to the β-carbon releases acetyl-CoA and completes the cycle. The resultant acyl-CoA can start at step (1) and proceed through the cycle until it is completely degraded to acetyl-CoA units.

d. Oxidation of fatty acids with an odd number of carbon atoms results in the production of one molecule of propionyl-CoA from the ω-end of the fatty acid. This molecule is released during the final thiolytic cleavage cycle. Propionyl-CoA can be carboxylated to methylmalonyl-CoA

IN A NUTSHELL

β-Oxidation of fatty acids:

- *Occurs in mitochondria*
- *Oxidizes β-carbon atom*
- *α-oxidation occurs only in brain*
  - *absence of α-oxidation → Refsum's disease*
- *ω-oxidation in liver (minor pathway)*
- *Activation uses 2 ATP per molecule*
- *Carnitine transports fatty acid from the cytosol to the mitochondria*
- *Oxidation removes 2 carbons per cycle*
  - *1 acetyl-CoA in TCA cycle*
  - *1 $FADH_2$ and 1 NADH → 5 ATP*
- *Odd-carbon fatty acids*
  - *propionate (C_3) is carboxylated to methylmalonyl-CoA (C_4)*
  - *methymalonyl-CoA + vitamin B_{12} → succinyl-CoA → TCA cycle*
- *Unsaturated fatty acids*
  - *cis bonds → trans bonds, then oxidation continues*

and then converted to succinyl-CoA for entry into the TCA cycle for further metabolism. The oxidation of unsaturated fatty acids is also important, since nearly 50% of human fatty acids are unsaturated. Unsaturated fatty acids in natural fat contain cis double bonds only, but the enzyme enoyl-CoA hydratase, which catalyzes the second reaction in the β-oxidation cycle, acts only on trans double bonds. An auxiliary enzyme, cisΔ^3-transΔ^2 enoyl-CoA isomerase, converts natural cis bonds to the trans configuration, allowing continued oxidation of the unsaturated fatty acid.

2. **Energetics of β-oxidation.** Oxidation of palmitic acid (C_{16}) produces 8 molecules of acetyl-CoA, 7 molecules of NADH, and 7 molecules of $FADH_2$. Further oxidation of acetyl-CoA by the TCA cycle results in the following stoichiometry for the oxidation of palmitic acid:

$$C_{16}H_{32}O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16\ H_2O$$

The net yield of usable energy, therefore, from the complete oxidation of one molecule of palmitic acid is 129 molecules of ATP. Since the respiratory quotient (RQ) is defined as moles of CO_2 produced divided by moles of O_2 consumed, the RQ for fatty acid oxidation is approximately 0.7. In contrast, the RQ for the complete oxidation of glucose via glycolysis and the TCA cycle is approximately 1.0.

3. **Regulation of fatty acid oxidation** is achieved by controlling the rate at which fatty acids enter the mitochondria. CAT-I is inhibited by malonyl-CoA, the first compound committed to fatty acid synthesis. This ensures that a futile cycle is not generated by simultaneous synthesis and oxidation of fatty acids. The rate of oxidation is also controlled by the rate at which fatty acids are released from triacylglycerol stores in adipose tissue.

4. **Comparison of fatty acid synthesis and oxidation.** The key differences between synthesis and oxidation of fatty acids are summarized in Table 5-6.

C. **Ketone body metabolism.** The major ketone bodies are **acetoacetic** and **β-hydroxybutyric acids**, weak acids that dissociate to acetoacetate and β-hydroxybutyrate at physiological pH. Spontaneous decarboxylation of acetoacetate gives rise to acetone. These compounds are synthesized from acetyl CoA by mitochondria when excessive amounts of fatty acids are being oxidized and glucose availability is limited. Ketone synthesis is inhibited when adequate carbohydrate is available. Ketone bodies are used as fuel by extrahepatic tissues.

NOTE

Oxidation of fatty acids produces more energy than carbohydrate oxidation, with 9 kcal/g of fat vs. 4 kcal/g of glucose.

CLINICAL CORRELATE

Ketosis, the overproduction of ketones, occurs as a result of a high glucagon/insulin ratio during carbohydrate deficiency states, such as starvation, severe diabetes, and alcoholism. The acidic properties of the ketones lower the pH of the plasma, causing metabolic acidosis and decreased plasma bicarbonate levels.

	Synthesis	β-Oxidation
Final product	Palmitate	Acetyl-CoA
Tissues involved	Adipose, liver	Muscle, liver
Subcellular localization	Cytoplasmic	Mitochondrial
Membrane transport	Citrate shuttle carries acetyl groups from mito matrix into cytosol	Carnitine shuttle carries fatty acids from cytosol into mito matrix
Cofactors	NADPH	NAD^+, FAD
Effect of high insulin/glucagon ratio	Pathway preferred	Pathway suppressed
Other regulators	Activated by citrate	Inhibited by malonyl-CoA
Rate-limiting enzyme	Acetyl-CoA carboxylase	CAT-I

Table 5-6. Comparison between synthesis and oxidation of fatty acids.

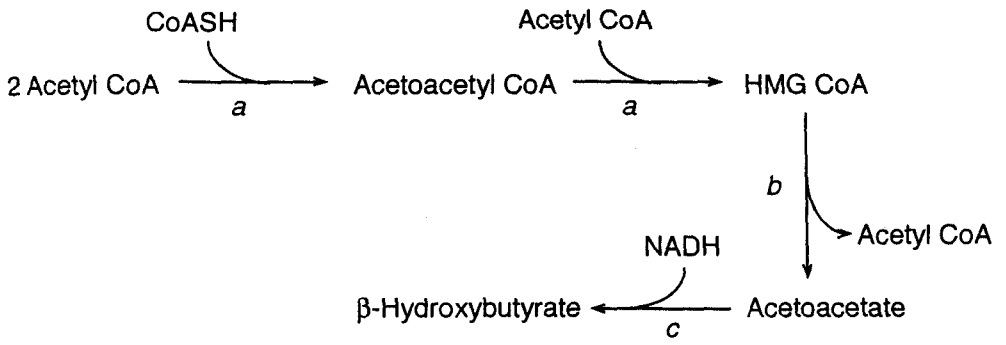


Figure 5-16. Ketone synthesis.

1. **Ketone synthesis** occurs in liver mitochondria via the reactions shown in Figure 5-16.
   a. Condensation of three molecules of acetyl-CoA in two sequential reactions generates β-hydroxy-β-methylglutaryl-CoA (HMG-CoA). Acetoacetyl-CoA is formed in the first reaction and acts as the substrate for the second.
   b. Cleavage of HMG-CoA produces acetoacetate and acetyl-CoA.
   c. Reduction of acetoacetate to β-hydroxybutyrate produces NADH.
2. **Ketone utilization** by extrahepatic tissues occurs in the mitochondria. Following the uptake of ketones, β-hydroxybutyrate is oxidized back to acetoacetate. The fate of acetoacetate involves two sequential reactions as shown in Figure 5-17.
   a. Conversion of acetoacetate to acetoacetyl-CoA is catalyzed by an enzyme that transfers CoA-SH from succinyl-CoA to acetoacetate. The absence of this transferase in liver accounts for the inability of liver to use ketones as fuel.
   b. Thiolytic cleavage of acetoacetyl-CoA releases two molecules of acetyl-CoA. This reaction is catalyzed by thiolase, an enzyme of the β-oxidation pathway.

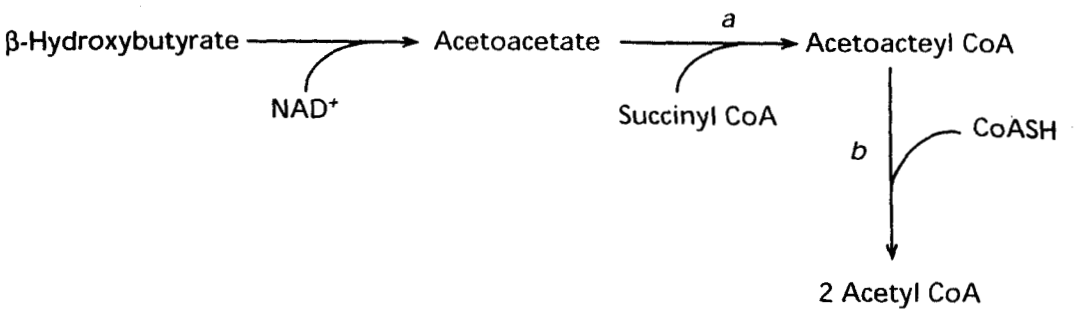


Figure 5-17. Ketone utilization.

NOTE

The fruity breath odor commonly seen in patients with ketosis is caused by the ventilation of acetone (produced by the decarboxylation of acetoacetone).

D. **Triacylglycerol metabolism.** Triacylglycerols are the most common storage and transport form for fatty acids derived from either dietary sources or *de novo* synthesis. The synthesis and degradation of triacylglycerols involves formation and hydrolysis of the ester bonds between fatty acids and the glycerol backbone. The conditions that promote lipogenesis are a high-carbohydrate diet and elevated insulin/glucagon ratio. Fasting, exercise, and a decreased insulin/glucagon ratio promote lipolysis.

1. **Triacylglycerol synthesis** occurs primarily in liver and adipose tissue. Synthesis requires glycerol phosphate, which is formed by the reduction of dihydroxyacetone phosphate (DHAP), an intermediate in glycolysis. Fatty acids are transferred from fatty acyl-CoA to carbons 1 and 2 of the glycerol backbone by fatty acyl transferase. Hydrolysis of the phosphate bond at carbon-3 generates **diacylglycerol,** which is then esterified with a third fatty acid, producing **triacylglycerol**. A summary of triacylglycerol synthesis is shown in Figure 5-18.

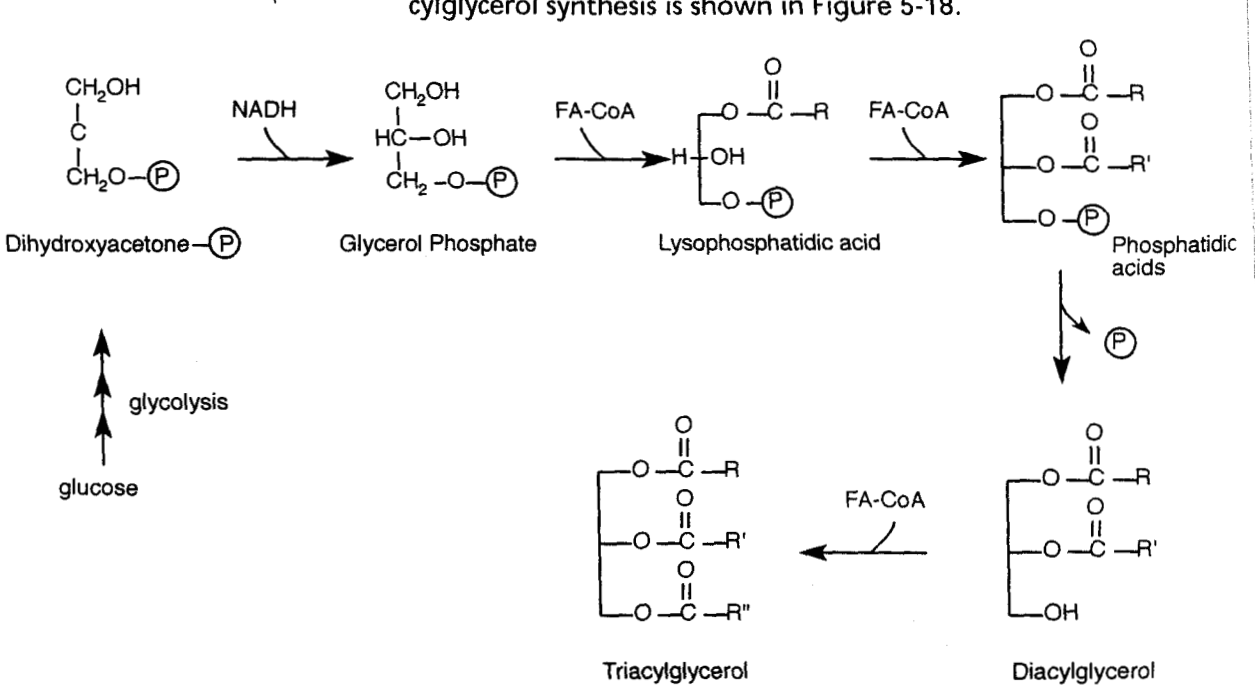


Figure 5-18. Triacylglycerol synthesis.

2. **Triacylglycerol hydrolysis** involves a family of lipases that differ in their localization and specificity. A summary of triacylglycerol hydrolysis is shown in Figure 5-19.
   a. **Hormone-sensitive lipase** is found primarily in adipose tissue where it hydrolyzes the first fatty acid from triacylglycerol. This is the rate-limiting step in mobilization of fatty acids from adipose stores. The enzyme is activated by cAMP-dependent phosphorylation. A number of hormones, including epinephrine and norepinephrine, stimulate cAMP production in adipose tissue and initiate lipolysis. The fatty acids released from adipose are carried in the plasma bound to albumin. Glycerol is released and extracted by the liver, where it can be used for gluconeogenesis or resynthesis of triacylglycerol. The adipocyte cannot recycle glycerol due to the absence of glycerol kinase.

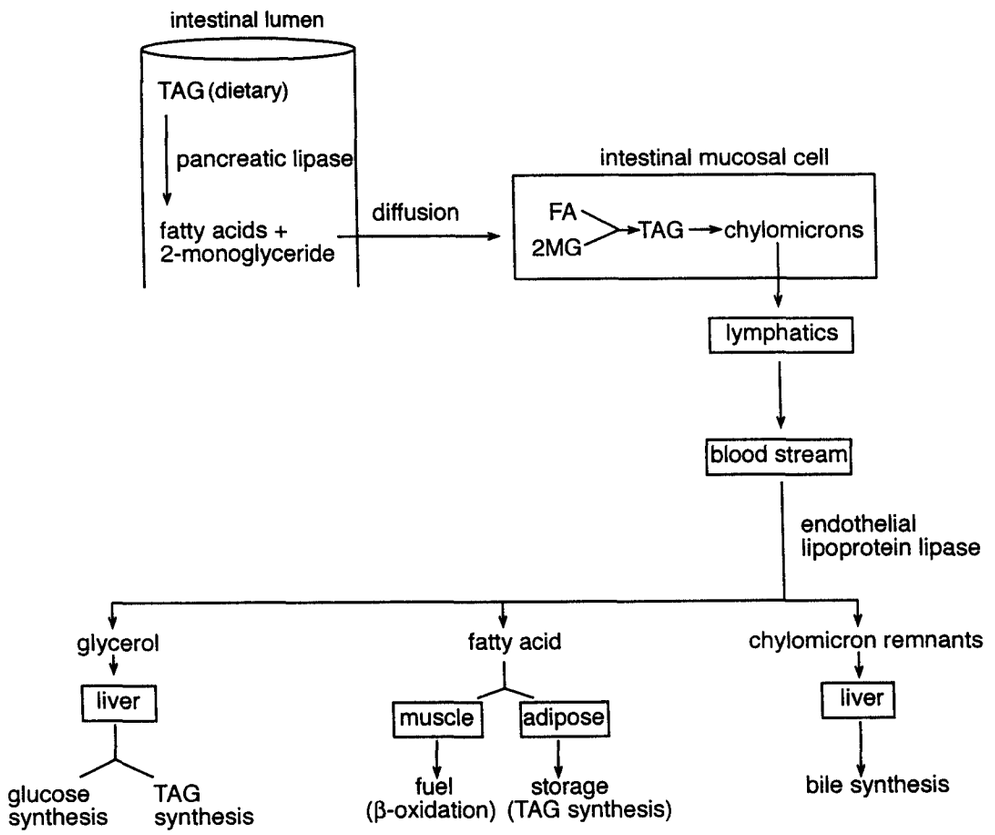


Figure 5-19. Summary of triacylglycerol hydrolysis.

b. **Pancreatic lipase** hydrolyzes dietary triacylglycerol in the small intestine. Hydrolysis occurs at positions 1 and 3, resulting in the release of two molecules of fatty acid, and 2-monoglyceride. These compounds diffuse into the intestinal mucosal cells, where they are re-esterified to triacylglycerol and incorporated into chylomicrons. The chylomicrons are released into the lymphatics and enter the bloodstream.

c. **Lipoprotein lipase (LpL)** is produced by the endothelial cells of the vasculature in adipose and muscle tissue. It hydrolyzes chylomicrons and VLDL triacylglycerol into free fatty acids and glycerol. The fatty acids are taken up by adipose and muscle tissue. In adipose tissue they are reesterified into triacylglycerols for storage. In muscle, the free fatty acids are oxidized for energy. The glycerol produced by the action of lipoprotein lipase is taken up by liver. The chylomicron remnants, enriched in cholesterol, are taken up by the liver where the cholesterol can be converted to bile acids or repackaged into VLDLs.

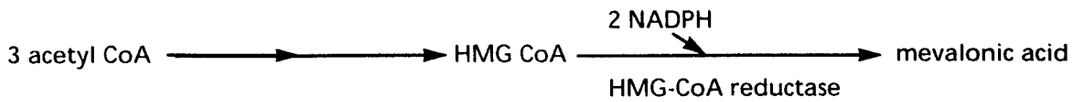
CHOLESTEROL METABOLISM

There are two routes by which cholesterol is introduced into the body: it can originate from the diet, or it can be synthesized *de novo* by any nucleated cell. Cholesterol is an integral structural component of plasma membranes and serves as a precursor of vitamin D, bile acids, and steroid hormones.

A. **Cholesterol synthesis.** The major site of cholesterol synthesis is the liver. The adrenal cortex, the ovaries, and the testes also produce significant amounts of cholesterol and use it for steroid hormone synthesis. The enzymes required to synthesize cholesterol are extramitochondrial and are localized in either the cytosol or the endoplasmic reticulum. The substrates required for cholesterol synthesis are acetyl-CoA, NADPH, ATP, and O_2. The overall equation for cholesterol synthesis is:

$$18 \text{ acetyl CoA} + 18 \text{ NADPH} + 18 \text{ ATP} + 4\ O_2 \rightarrow \text{cholesterol } (C_{27}) + 18\ NADP^+ + 18 \text{ ADP} + 18\ P_i + 9\ CO_2$$

1. **Stages of cholesterol synthesis.** The synthetic pathway occurs in four stages.
   a. **Mevalonic acid synthesis.** The first stage of cholesterol synthesis is the sequential condensation of three molecules of acetyl-CoA to produce HMG-CoA, which is then reduced to mevalonic acid.



The key enzyme in the overall pathway is **HMG-CoA reductase**. This enzyme reduces HMG-CoA to mevalonic acid in a reaction that requires two molecules of NADPH. This is the rate-limiting step in cholesterol synthesis and is therefore the site at which synthesis is controlled. The synthesis of HMG-CoA involves the same steps as were described for ketone synthesis with two exceptions: the synthesis of HMG-CoA in the cytosol and mitochondria require different sets of enzymes, and the cytosolic HMG-CoA pool used for cholesterol synthesis does not mix with the mitochondrial pool used for ketone synthesis.

b. **Synthesis of activated isoprene units.** The second stage of cholesterol synthesis is the conversion of mevalonic acid (C_6) into activated isoprene units (C_5) that are designed to condense with one another. Two types of activated isoprene units are synthesized: isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DPP). These compounds have many synthetic uses, including acting as substrates for dolichol, ubiquinone, and fat-soluble vitamin synthesis. Synthesis of an activated isoprene unit requires three molecules of ATP.

Isopentenyl Pyrophosphate

3,3 Dimethylallyl Pyrophosphate

Figure 5-20. Activated isoprene units.

c. **Formation of squalene.** In the third stage, squalene, a C_{30} linear compound, is formed by condensation reactions that use six activated isoprene units.
Squalene is generated via three condensation reactions:
(1) Condensation of two C_5 units.

$$\text{IPP} + \text{DPP} \rightarrow \text{geranyl pyrophosphate} + PP_i$$

(2) Condensation of C_5 and C_{10} units.

$$\text{IPP} + \text{geranyl-PP} \rightarrow \text{farnesyl pyrophosphate} + PP_i$$

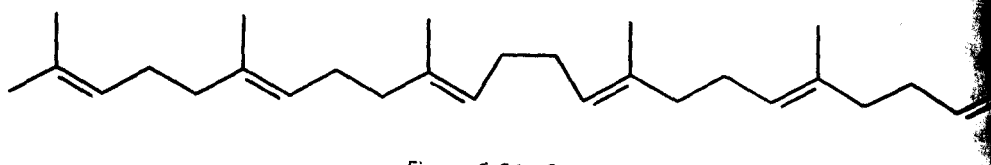


Figure 5-21. Squalene.

(3) Condensation of two C_{15} units.

$$\text{farnesyl-PP} + \text{PP-farnesyl} \rightarrow \text{squalene} + PP_i$$

d. **Conversion of squalene to cholesterol.** The fourth and final stage of cholesterol synthesis involves the cyclization of squalene to form lanosterol, which is converted via a number of reactions to form cholesterol (Figure 5-22).

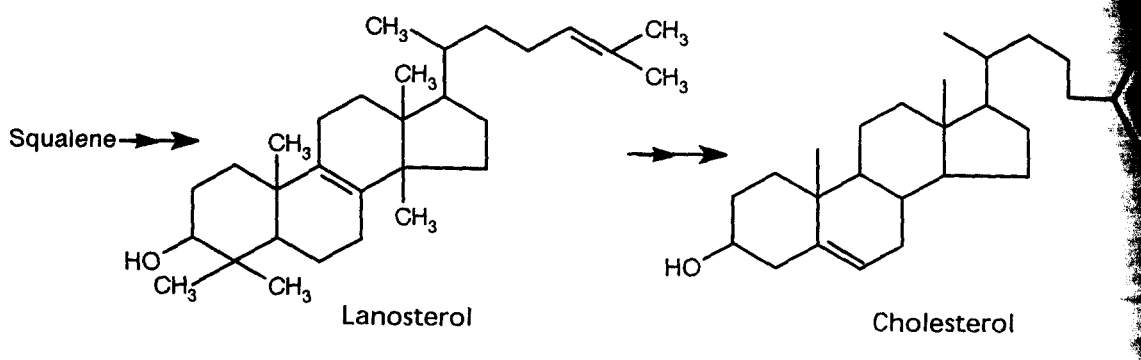


Figure 5-22. Conversion of squalene to cholesterol.

2. **Cholesterol ester hydrolysis and formation.** Approximately 70 percent of the cholesterol found in humans exists as cholesterol ester. The most common fatty acid found esterified to the 3-hydroxyl group of the A ring of cholesterol is **oleic acid.**
   a. **Hydrolysis of cholesterol esters**
      (1) **Pancreatic cholesterol esterase** hydrolyzes dietary cholesterol esters in the intestinal lumen. Re-esterification occurs after diffusion into the intestinal mucosal cell, but prior to incorporation into chylomicrons.
      (2) **Intracellular cholesterol esterases** are found in all tissues, but are in highest concentrations in liver and steroid hormone-producing glands.
   b. **Synthesis of cholesterol esters.** Two enzymes contribute to the synthesis of cholesterol esters.
      (1) **Lecithin cholesterol acyl transferase (LCAT)**, found in the plasma, participates in reverse cholesterol transport by HDL. Esterification involves the transfer of a fatty acid from the 2-position of phosphatidylcholine to cholesterol.

(2) **Acyl cholesterol acyl transferase (ACAT)**, found inside the cell, is involved in cholesterol storage. Esterification involves the transfer of a fatty acid from fatty acyl-CoA to cholesterol.

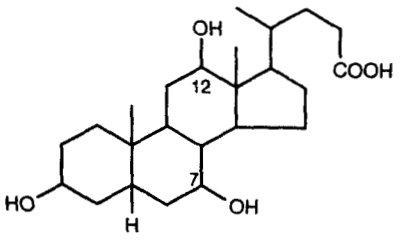
3. **Regulation of cholesterol synthesis.** Cholesterol synthesis is highly regulated. Diets high in fat and carbohydrate stimulate HMG-CoA reductase, the rate-limiting enzyme in the pathway. The activity is suppressed by high dietary cholesterol and by fasting, which limits the available acetyl-CoA and NADPH required for synthesis. HMG-CoA is also under hormonal control: it is stimulated by insulin and thyroxine, and inhibited by glucagon. Finally, cholesterol is constantly recycled between hepatic and extrahepatic tissues, either via the enterohepatic bile circulation or from cholesterol-rich chylomicron remnants, IDLs, and LDLs. These lipoprotiens bind to specific receptors on cell membranes and are internalized and hydrolyzed, yielding free cholesterol. This increase in free cholesterol in the cell has two major regulatory effects: it suppresses the synthesis of LDL receptors ("down regulation"), thereby decreasing additional uptake of LDL-cholesterol from plasma and it suppresses the synthesis of HMG-CoA reductase, thus decreasing the *de novo* synthesis of cholesterol by the cells.

B. **Cholesterol derivatives.** Cholesterol is the precursor for bile acid and steroid hormone synthesis.

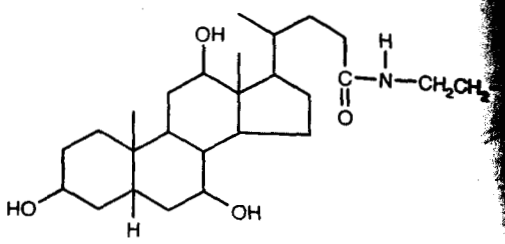
1. **Bile acids** are 24-carbon compounds derived from cholesterol. Synthesis occurs in the liver, and involves four reactions, each of which make bile acids more amphipathic and more suitable for their roles in lipid emulsification. Bile acids are the major excreted form of cholesterol.
   a. **Side chain cleavage of cholesterol** releases a three-carbon fragment and results in a C_{24} compound in which the terminal carbon of the side chain has been oxidized to an acid.
   b. **Conjugation** of the side chain carboxyl group with either glycine or taurine adds a more acidic group and ensures complete ionization at physiologic pH.
   c. **Reduction** of the double bond in the ring system uses NADH as a source of reducing power.
   d. **Hydroxylation** at carbons 7 and 12 is catalyzed by microsomal mixed-function hydroxylases in reactions that require both O_2 and NADPH. An example of a bile acid (cholic acid) and its conjugated derivative (taurocholic acid) are shown in Figure 5-23. The term "bile salt" indicates that at physiologic pH, the side chain group is negatively charged and is associated with a counterion.

MNEMONIC

*LCAT is involved in the **l**eaving of cholesterol via HDL; **A**CAT is involved in the **a**ccumulation of cholesterol in cells*



Cholic acid

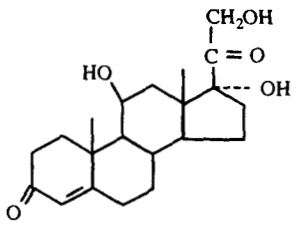


Taurocholic acid

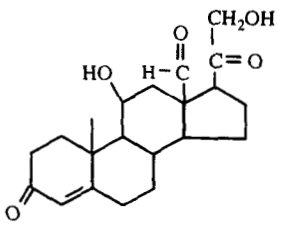
Figure 5-23. Cholic acid and taurocholic acid.

2. **Steroid hormones** are derived from cholesterol in the adre cortex, the ovaries, and the testes. The structures of the ma steroid hormones are shown in Figure 5-24. Steroid hormo synthesis involves two reactions that are common to all path ways, and numerous additional reactions that have tissue an cell specificity.

Adrenal Cortical Steroids

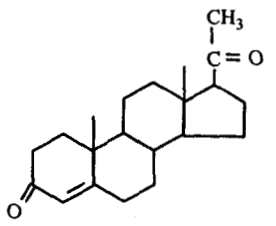


Cortisol

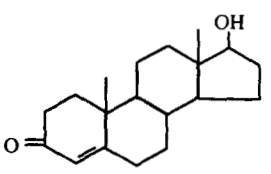


Aldosterone

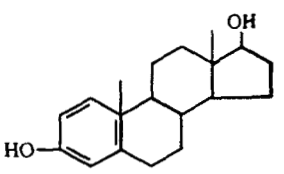
Gonadal Steroids



Progesterone



Testosterone



17β-Estradiol

Figure 5-24. Major steroid hormones.

a. **Common synthetic reactions.** The initial reaction in the synthesis of all steroid hormones is cleavage of the side chain to generate **pregnenolone,** a C_{21} intermediate. Pregnenolone is converted to **progesterone** by the action of **3-β-hydroxysteroid dehydrogenase** (3-β-HSD). Both pregnenolone and progesterone are C_{21} intermediates in the synthesis of all steroid hormones. The rate-limiting step

in steroid hormone synthesis is the initial cleavage of the side chain. The enzyme **20-22 desmolase** is located in the mitochondria and its activity is increased in response to hormone binding to membrane receptors. In cells that synthesize glucocorticoids, ACTH stimulates desmolase activity; in mineralocorticoid synthesis, the activity is stimulated by angiotensin II; in androgen and estrogen synthesis, the activity is stimulated by LH.

b. **Tissue-specific synthetic reactions** include the following four types of reactions.
   (1) **Hydroxylation reactions** can occur at various positions on the steroid nucleus. Cortisol synthesis by the adrenal cortex requires hydroxylation at carbons 11, 17, and 21, whereas aldosterone synthesis requires hydroxylation at carbons 11, 18, and 21.
   (2) **Side chain cleavage by 17-20 desmolase** removes the remainder of the side chain and converts the steroid to a C_{19} androgen.
   (3) **5-α-reductase** reduces the double bond in testosterone to form dihydrotestosterone (DHT). This enzyme is present in tissues that use DHT as the major androgen.
   (4) **Aromatase** removes the methyl group that extends up between the A and B rings of the steroid nucleus and makes the A ring aromatic. This enzyme is present in tissues that convert androgens to estrogens.

PHOSPHOLIPIDS

Phospholipids are the major building blocks of membranes. They also participate in signal transduction pathways and serve as reservoirs for polyunsaturated fatty acids needed for eicosanoid synthesis.

A. **Synthesis.** The synthesis of phosphatidylethanolamine (PE) will be used to illustrate the synthetic strategy used to form these compounds. The two major substrates required for PE synthesis are: activated ethanolamine and diacylglycerol. Activation of ethanolamine occurs in a two-step reaction. Free ethanolamine is first phosphorylated and then condensed with cytidine triphosphate to generate CDP-ethanolamine (Figure 5-25). The formation of **phosphatidylethanolamine** involves the transfer of P-ethanolamine from CDP-ethanolamine to the free hydroxyl group of diacylglycerol (Figure 5-26).

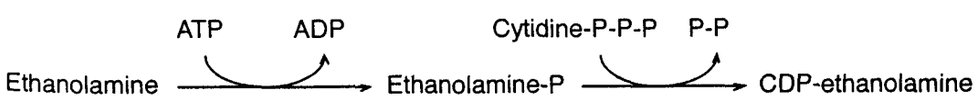


Figure 5-25. Activation of ethanolamine.

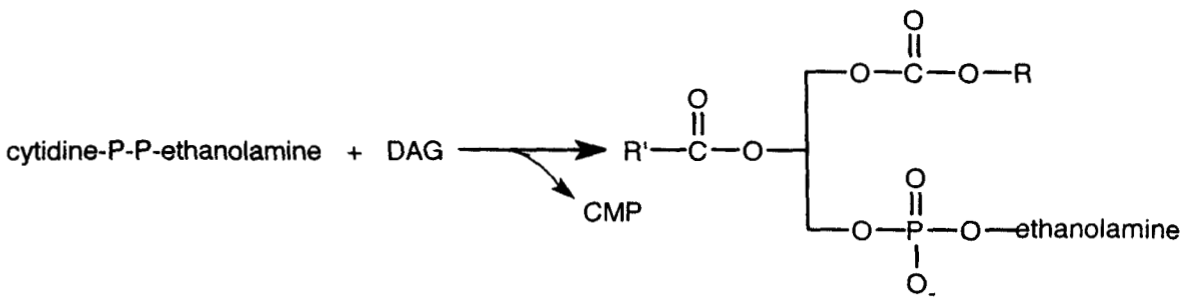


Figure 5-26. Formation of phosphatidylethanolamine.

IN A NUTSHELL

Phospholipid synthesis:

- *ATP phosphorylates ethanolamine*
- *CTP activates phosphoethanolamine*
- *CDP-ethanolamine combines with diacylglycerol to form phosphatidylethanolamine*

B. **Interconversion of phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylcholine (PC).** Three types of reactions allow interconversion between these three classes of phospholipids.

1. **Polar head group exchange**. The ethanolamine moiety of phosphatidylethanolamine can undergo an exchange with free serine, resulting in phosphatidylserine.
2. **Decarboxylation** of phosphatidylserine results in phosphatidylethanolamine.
3. **Methylation** of phosphatidylethanolamine in three consecutive reactions results in phosphatidylcholine. S-adenosylmethionine (SAM) is used as a methylating agent. These interconversions are illustrated in Figure 5-27.

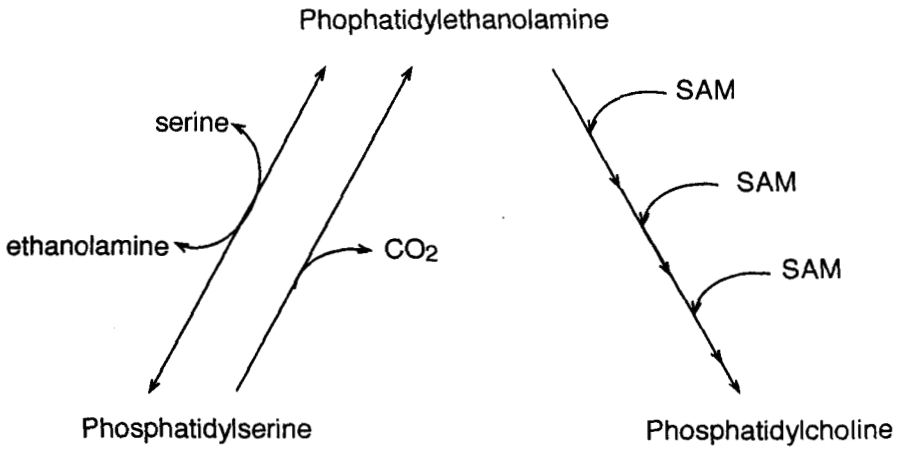


Figure 5-27. Interconversion of PE, PS, and PC.

C. **Phosphatidylinositol synthesis** uses a variation of the above pathway in which the diacylglycerol moiety is activated by the formation of CDP-diacylglycerol. DAG-phosphate is subsequently transferred from CDP-DAG to one of the hydroxyl groups of inositol.

SPHINGOLIPIDS AND GLYCOLIPIDS

This group of lipids is classified into five major categories: **sphingomyelin, cerebrosides, sulfatides, globosides,** and **gangliosides**. All five classes are categorized as sphingolipids, containing **ceramide** (N-acyl-sphingosine). Additionally, all of the classes except sphingomyelin are glycolipids due to the presence of carbohydrate.

A. **Synthesis of sphingolipids and glycolipids** occurs in two major steps. The first step is the synthesis of the ceramide core. The common ceramide core is synthesized from three precursors: palmitoyl-CoA, serine, and a long-chain fatty acyl-CoA. All classes of sphingolipids and glycolipids are formed by the transfer of groups from their activated carriers to a hydroxyl group on the terminal carbon of ceramide. Activated carriers used in sphingolipid and glycolipid synthesis include UDP-sugars, CDP-choline, phosphoadenosine phosphosulfate (PAPS), and CMP-N-acetylneuraminic acid (CMP-NANA).

B. **Classes of sphingolipids and glycolipids**

1. **Sphingomyelin** is a major component of membranes of the central nervous system. It is the only sphingolipid that contains phosphate. The transfer of phosphocholine from UDP-choline to ceramide forms sphingomyelin.
2. **Cerebrosides and sulfatides.** Cerebrosides contain either glucose or galactose. The addition of sulfate to galactocerebroside generates a sulfatide. Synthesis requires UDP-glu, UDP-gal, and PAPS as "active sulfate."
3. **Globosides.** The addition of two or more sugars to ceramide results in globosides. The sugars may be glucose, galactose, or N-acetylgalactosamine. These compounds are important constituents of red blood cell membranes.
4. **Gangliosides.** Glycolipids containing **neuraminic acid,** the most common form of sialic acid, are classified as gangliosides. These lipids are found in very high concentration in ganglion cells of the central nervous system.

Disease	Mode of inheritance	Mechanism of disease	Characteristics
Gaucher's	Autosomal recessive	Deficiency of β-glucocerebrosidase ↑ Glucocerebrosides in brain, liver, spleen, bone marrow	Hepatosplenomegaly Neurologic deficits Mental retardation
Fabry's	X-linked recessive	Deficiency of α-galactosidase ↑ Ceramide trihexoside	Renal failure Telangiectasias Skin rash Pain in lower extremities
Tay-Sachs	Autosomal recessive	Deficiency of hexosaminidase A ↑ GM_2 gangliosides	Mental retardation, blindness, muscular weakness Cherry red spot on macula Death by age 3 Common in Ashkenazic Jews
Niemann-Pick	Autosomal recessive	Deficiency of sphingomyelinase ↑ Sphingomyelin and cholesterol in reticuloendothelial tissues	Mental retardation Hepatosplenomegaly Neurologic deficits Foam cells in bone marrow Cherry red spot in 40% of cases Death by age 3
Farber		Deficiency of ceramide ↑ Ceramide	Hoarseness, dermatitis Skeletal deformations Mental retardation Hepatomegaly

Table 5-7. Common sphingolipid disorders.

C. **Sphingolipidoses** (Table 5-7)

PROSTAGLANDINS, THROMBOXANES, AND LEUKOTRIENES

These compounds, collectively known as the **eicosanoids,** are derived from **arachidonic acid** and play crucial regulatory roles in human physiology. They bind to cell surface receptors and act as autocrine and paracrine hormones. They exert their effects at very low concentrations, have extremely short half-lives, and have many diverse functions (Table 5-8).

A. **Prostaglandins** are 20-carbon hydroxy fatty acids containing a five-membered ring. **Prostacyclin**, PGI_2, is the major prostaglandin and is synthesized by endothelial cells. It promotes vasodilatation of the coronary arteries and antagonizes platelet aggregation.

B. **Thromboxanes** are 20-carbon hydroxy fatty acids with a six-membered ring containing an oxygen atom. **Thromboxane A_2** is the

major thromboxane and it antagonizes prostacyclin, leading to platelet aggregation and vasoconstriction. It is synthesized primarily by platelets.

C. **Leukotrienes** contain no ring. They play a major role in inflammatory responses and chemotaxis. The **slow-reacting substance of anaphylaxis (SRS-A)** is a leukotriene that acts as a potent smooth muscle constrictor.

D. **Synthesis of prostaglandins, thromboxanes, and leukotrienes.** Arachidonic acid (AA) is the major precursor of these compounds. AA is stored in membrane phospholipids, where it is esterified to carbon-2 of the glycerol backbone. The release of AA from the membrane is catalyzed by the action of **phospholipase A_2.**

Enzyme	Action
Phospholipase A_2	Releases arachidonic acid from membrane stores; inhibited by glucocorticoids (e.g., cortisone)
Cyclooxgenase	Catalyzes initial step in the pathway that converts arachidonic acid to prostaglandins and thromboxanes; inhibited by nonsteroidal anti-inflammatory drugs
Lipoxygenase	Catalyzes the initial step in the pathway that converts arachidonic acid to leukotrienes

Table 5-8. Key enzymes in eicosanoid metabolism.

Amino Acid Metabolism

One of the major functions of the amino acid pool is to provide a source of building blocks for protein synthesis. Humans can synthesize only ten of the twenty amino acids found in proteins. The remainder are termed the "essential" amino acids, and must be supplied by dietary protein. Both the quantity and the composition of the amino acid pool in cells remain remarkably constant under a wide variety of conditions. Processes that contribute to the pool of amino acids include degradation of body protein, digestion and absorption of dietary protein, and the *de novo* synthesis of nonessential amino acids. Processes that remove amino acids from the pool include the synthesis of body protein, the oxidation of excess amino acids to CO_2 and H_2O, and the synthesis of a large number of specialized products, including heme, neurotransmitters, and catecholamines. This chapter will review protein digestion and absorption, pathways for synthesis of nonessential amino acids, mechanisms for disposing of α-amino groups (urea synthesis and ammoniagenesis) and the "remnant" carbon skeletons, and the use of amino acids as precursors for a large number of specialized products.

ESSENTIAL AMINO ACIDS

An essential amino acid is one that cannot be synthesized in adequate amounts to meet the needs of the cell, and therefore must be supplied by dietary protein. There are ten essential amino acids (Table 6-1). Two of these (arginine and histidine) are essential only in infants and children.

MNEMONIC

In sentence form:

Any help in learning these little molecules proves truly valuable

(arg, his, ile, leu, thr, lys, met, phe, trp, val)

In name form:

***Pvt. Tim Hall,** US Army*

(phe, val, thr, trp, ile, met, his, arg, leu, lys)

Essential amino acids	
Arginine*	Methionine
Histidine*	Phenylalanine
Isoleucine	Threonine
Leucine	Tryptophan
Lysine	Valine

*Essential only in early childhood

Table 6-1. Essential amino acids.

Humans are unable to synthesize the aromatic ring system in amino acids, the branched chain amino acids, or the basic amino acids.

A. **The amino acid pool**. There are approximately 100 grams of free amino acids in the body, of which nearly 50% is glutamine and alanine. Approximately 10% of the total pool consists of essential amino acids. The major drain on the amino acid pool is protein synthesis, with 200-300 grams of protein synthesized per day. In anabolic states, when insulin is acting on liver and muscle, this rate is increased. Additionally, specific amino acids are removed from the pool to support the synthesis of specialized compounds, including heme, neurotransmitters, creatine, polyamines, carnitine, and nucleotides. Therefore, an exogenous protein source is required to replenish and maintain the amino acid pool. The recommended protein requirement for a healthy adult is 0.8 grams/kg body weight/day.

B. **Digestion of dietary protein.** Protein digestion starts in the stomach, where the acidic gastric juice denatures the protein and activates **pepsin**. Proteins are hydrolyzed by pepsin to a mixture of large peptides. Further degradation occurs in the small intestine by the **pancreatic proteases**. These enzymes **(trypsin, chymotrypsin, elastase, carboxypeptidases)** are secreted as inactive precursors and are activated in the small intestine. Activation is initiated by **enteropeptidase,** an intestinal enzyme that converts inactive trypsinogen to trypsin. Trypsin, in turn, activates the other pancreatic proteases. These enzymes continue the hydrolysis of dietary protein, producing a mixture of free amino acids, dipeptides, and tripeptides.

C. **Absorption of amino acids.** Specific transport systems absorb amino acids and small peptides into the epithelial cells where the dipeptides and tripeptides are hydrolyzed to free amino acids before they leave the cell. The transport systems for amino acids in the intestinal cells are similar to those for glucose, where transport across the luminal membrane is Na^+-dependent and trans-

port across the contraluminal membrane is Na^+-independent. Several different carriers have been identified on the basis of their specificity. Genetic deficiencies in each of these have been reported. Hartnup disease and cystinuria are clinical problems arising from faulty transport mechanisms.

1. **Hartnup disease** is a rare autosomal recessive disorder characterized by the excretion of large quantities of neutral amino acids in the urine. The clinical symptoms of this disease can be attributed to the loss of tryptophan, a precursor of nicotinamide, in the urine. Similar symptoms are seen in pellagra, a condition due to niacin or nicotinamide deficiency. Up to 50% of nicotinamide is normally supplied by the metabolism of tryptophan.
2. **Cystinuria** is one of the most common amino acidurias. It is an autosomal recessive disease caused by a defect in the transport protein for lysine, arginine, cystine, and ornithine, resulting in their excretion in the urine. The low solubility of cystine leads to the precipitation and formation of kidney stones.

MNEMONIC

Cystinuria results from an inability to reabsorb COAL in the renal tubules: ***c****ystine,* ***o****rnithine,* ***a****rginine, and* ***l****ysine.*

AMINO ACID DEGRADATION

The catabolism of amino acids will be reviewed in two parts: the disposal of the α-amino group and the disposal of the carbon skeletons. Many of these reactions require the actions of **pyridoxal phosphate.**

A. **Disposal of α-amino groups.** The degradation of amino acids usually begins with removal of the α-amino group. Approximately 85% of the amino nitrogen ultimately gets excreted as urea. The two most important reactions in the flow of nitrogen from amino acids to urea are **transaminations** and the **oxidative deamination of glutamate.** All of the amino acids except lysine and threonine have specific transaminases that are involved in the disposal of the α-amino group.
   1. **Transaminases** are a family of enzymes that transfer the α-amino group from an amino acid to an acceptor, an α-keto acid. Pyridoxal phosphate (PLP) acts as a transient carrier in the reaction (Figure 6-1). Transaminases are specific for the amino acid, but are nonspecific for the acceptor. Most transaminases use **α-ketoglutarate** as an acceptor, providing a mechanism for funneling the amino groups from many amino acids into a common pool of **glutamate.**
   2. **Fate of glutamate.** Two routes for disposal of the α-amino group of glutamate exist in the liver. It may be released as ammonia or transferred to oxaloacetate.

NOTE

Pyridoxal phosphate is a derivative of pyridoxine (vitamin B_6) and acts as a coenzyme for all transaminases.

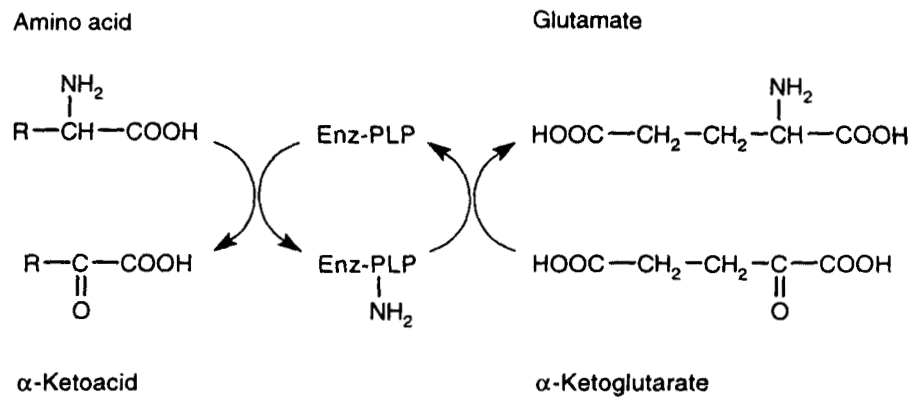


Figure 6-1. Transfer of α-amino groups from amino acids to glutamate.

a. **Oxidative deamination.** This reaction is catalyzed by **glutamate dehydrogenase,** a mitochondrial enzyme that releases an ammonium ion from glutamate, making it available for urea synthesis.

$$\text{Glutamate} + NAD^+ \xleftarrow[\text{Glutamate Dehydrogenase}]{} \alpha\text{-Ketoglutarate} + NADH + NH_4^+$$

b. **Transamination to oxaloacetate.** Some of the α-amino groups that have been collected in glutamate are transferred to oxaloacetate to form aspartate. Aspartate, in turn, acts as a donor of amino groups in the synthesis of urea. This reaction occurs mainly in the cytosol of liver, and is catalyzed by **aspartate transaminase (AST)**, also known as glutamate:oxaloacetate transaminase.

$$\text{Glutamate} + \text{Oxaloacetate} \xleftarrow[\text{Aspartate Dehydrogenase}]{} \alpha\text{-Ketoglutarate} + \text{Aspartate}$$

3. **Urea synthesis.** Urea synthesis occurs only in the liver. Urea contains two amino groups that are linked by a carbonyl group. One amino groups comes from an ammonium ion and the other is donated by aspartate. The carbonyl group comes from bicarbonate. The synthesis of one mol of urea requires five enzymes and three mols of ATP.

$$NH_4^+ + HCO_3^- + \text{Aspartate} + 3\ ATP \longrightarrow \text{Urea} + \text{Fumarate} + 2\ ADP + AMP + 2\ P_i + PP_i$$

a. **Reactions and enzymes of the urea cycle.** The first two steps in the urea cycle occur in the mitochondria, and the remaining steps occur in the cytosol. An overview of the urea cycle is shown in Figure 6-2, with a brief description of the enzymes and the reactions.

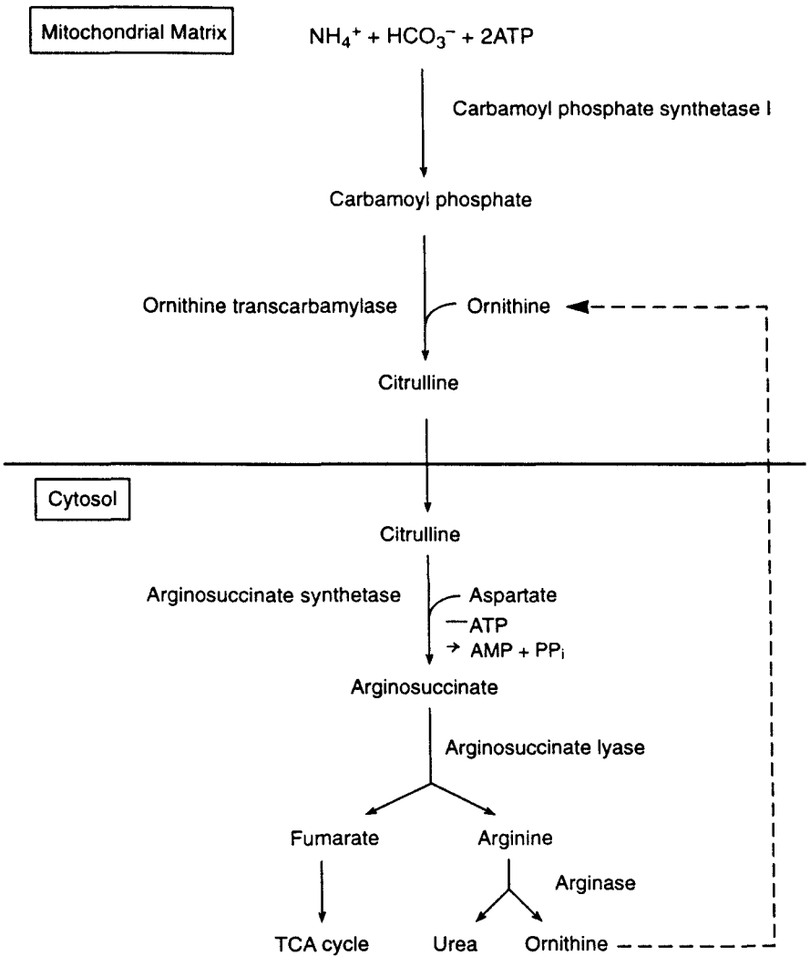


Figure 6-2. The urea cycle.

(1) **Carbamoyl phosphate synthetase I (CPS-I)** catalyzes the condensation of an ammonium ion and bicarbonate to form carbamoyl phosphate. The energy for creating the high-energy bond between bicarbonate and phosphate is supplied by two mols of ATP. This is the rate-limiting step in urea synthesis. The activity of CPS-I is allosterically activated by **N-acetylglutamate (NAG)**, a compound formed when glutamate accumulates in the mitochondria. This occurs when the diet is high in protein. The synthesis of NAG is activated by arginine. Carbamoyl phosphate synthetase II is a cytosolic enzyme that participates in pyrimidine synthesis. It uses glutamine as its nitrogen donor.

IN A NUTSHELL

High protein diet
↓
Glutamate accumulation
↓
Increase in NAG
↓
CPS-I activated

(2) **Ornithine transcarbamoylase (OTCase)** transfers the carbamoyl group from carbamoyl phosphate to ornithine, resulting in **citrulline.** Citrulline is then transported across the inner mitochondrial membrane into the cytosol, where the remaining reactions in the urea cycle occur.

(3) **Argininosuccinate synthetase** condenses citrulline with aspartate to form argininosuccinate. The α-amino group of aspartate is linked directly to citrulline in this reaction. The energy for forming the bond is provided by ATP hydrolysis to AMP and PP_i.

(4) **Argininosuccinate lyase** cleaves argininosuccinate to form arginine and fumarate. All of the aspartate molecule except the α-amino group is released as fumarate; the α-amino group becomes a part of the arginine side chain.

(5) **Arginase** releases urea from the side chain of arginine. The other product is **ornithine,** which is transported back into the mitochondria for participation in another cycle of urea synthesis. The urea is excreted in the urine. Note that urea contains two amino groups, one from aspartate and the other from ammonia.

b. **Abnormalities in the urea cycle.** Inherited deficiencies have been reported for each of the enzymes of the urea cycle. These can be distinguished from one another on the basis of the products excreted (Table 6-2). Ammonia accumulates in all of the deficiencies, but is most pronounced in deficiencies of CPS-I and OTCase.

NOTE

Arginase is found only in the brain, liver, and kidney.

Defective enzyme	Condition	Metabolites accumulated
Carbamoyl-P synthetase	Type I hyperammonemia	Ammonia, glutamine, alanine
Ornithine transcarbamoylase	Type II hyperammonemia	Ammonia, glutamine, orotate
Argininosuccinate synthetase	Citrullinemia	Citrulline
Argininosuccinate lyase	Argininosuccinic aciduria	Argininosuccinate
Arginase	Hyperargininemia	Arginine

Table 6-2. Defects in urea cycle enzymes.

IN A NUTSHELL

- *Amino groups in muscle are transferred to pyruvate to form alanine*
- *Alanine is picked up by liver, where it is converted back to pyruvate*
- *Liver uses pyruvate for gluconeogenesis and amino groups for urea synthesis*

4. **Alanine.** Skeletal muscle uses alanine as a carrier for transporting α-amino groups to the liver, where urea synthesis occurs. As shown in Figure 6-3, the amino groups that have been collected in glutamate are subsequently transferred to pyruvate with the formation of alanine. This reaction is catalyzed by **alanine aminotransferase** (also known as glutamate:pyruvate transaminase). Skeletal muscle releases large amounts of alanine into the circulation. Alanine is taken up by the liver,

where the reverse reaction occurs. This cycle allows amino groups from skeletal muscle to be transferred to the hepatic pool of glutamate. The alanine aminotransferases of both skeletal muscle and liver are cytosolic enzymes. Liver recycles pyruvate by using it for gluconeogenesis.

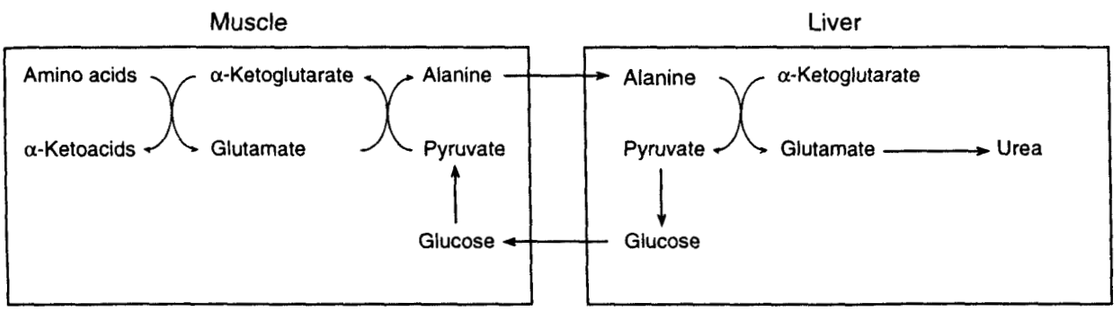


Figure 6-3. Alanine as a carrier of α-amino groups from muscle to liver.

5. **Glutamine.** All tissues produce some free ammonia that can be "detoxified" by the formation of glutamine. This reaction is important in extrahepatic tissues, where urea synthesis does not occur, and it is of particular importance in the brain, where the toxic effects of ammonia are severe.

$$\text{Glutamate} + NH_4^+ + \text{ATP} \xrightarrow{\text{Glutamine synthetase}} \text{Glutamine} + \text{ADP} + P_i$$

Skeletal muscle and brain release large amounts of glutamine into the circulation, with most of the glutamine being taken up by the kidney and the liver. The nitrogen is released from glutamine as ammonium ions in two sequential reactions catalyzed by glutaminase and glutamate dehydrogenase (Figure 6-4). Both of these reactions occur in mitochondria. In liver, the ammonia is incorporated into urea; in kidney, it is excreted as ammonium ion. Ammonia-genesis in kidney, is an important mechanism for titrating protons (60% of H^+ is excreted through NH_4^+). The activity of glutaminase is markedly increased in states of prolonged acidosis.

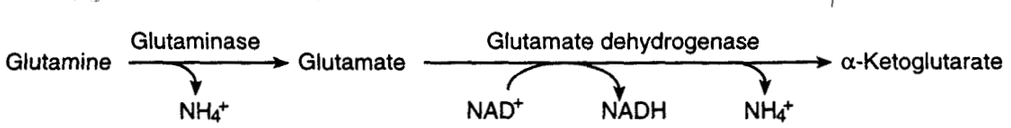


Figure 6-4. Glutamine as a carrier of amino groups.

B. **Disposal of carbon skeletons.** Carbon skeletons are the "remnants" of amino acids after the amino groups have been removed. The carbon skeletons can be oxidized to CO_2 and H_2O, with the generation of energy, or they can be used to synthesize

IN A NUTSHELL

- ***Strictly ketogenic:*** *leu, lys*
- ***Both ketogenic and glucogenic:*** *ile, phe, tyr, trp*
- ***Strictly glucogenic:*** *everything else*

either glucose or ketones. As shown in Figure 6-5, all of the amino acids can be degraded to seven common metabolites: acetyl-CoA, acetoacetyl-CoA, pyruvate, oxaloacetate, fumarate, succinyl-CoA, α-ketoglutarate, and propionyl-CoA (which is converted to succinyl-CoA). The ketogenic amino acids (leucine and lysine) are degraded to acetyl-CoA or acetoacetyl-CoA, both of which can be converted to ketone bodies. Four amino acids are both ketogenic and glucogenic (isoleucine, phenylalanine, tyrosine, and tryptophan). The remaining amino acids are strictly glucogenic.

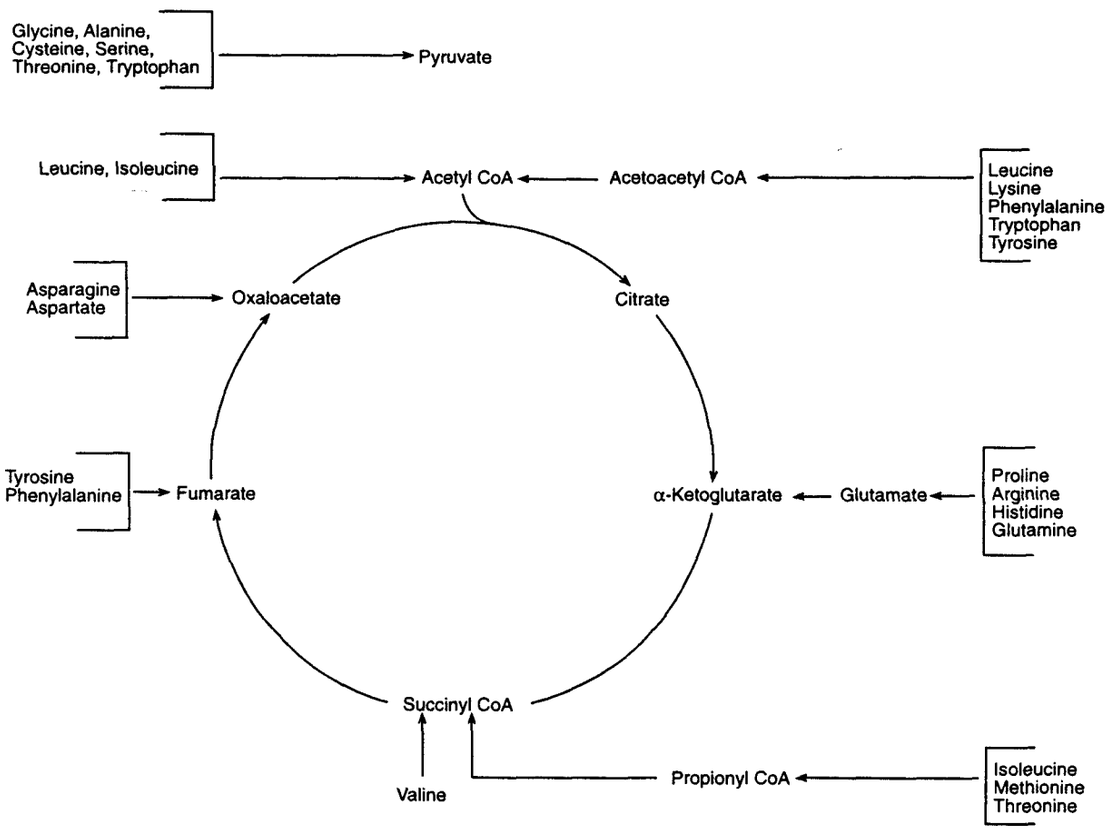


Figure 6-5. Metabolic intermediates from amino acids.

C. **Degradation of branched-chain amino acids.** Liver has little, if any, transaminase activity for valine, isoleucine, and leucine. Therefore, in contrast to the other amino acids, the degradation of the branched-chain amino acids starts in skeletal muscle, where transaminase activity is high. The first two reactions in the degradation of these three amino acids are catalyzed by common enzymes (Figure 6-6). **Branched-chain amino acid transaminase**

(BCAA transaminase) transfers the α-amino group to α-ketoglutarate to form glutamate and α-keto acids corresponding to the carbon skeletons valine, isoleucine, and leucine. This enzyme is present in high concentrations in skeletal muscle and very low concentrations in the liver. In the next step, the α-ketoacids are decarboxylated by a single **branched-chain ketoacid dehydrogenase (BCKA-DH),** resulting in branched-chain acyl-CoA analogs. The products of the BCKA-DH reaction proceed along different pathways. The end products for the degradation of each amino acid are shown in Figure 6-6. The end products of leucine and isoleucine are used by nerve tissue for the synthesis of lipid.

CLINICAL CORRELATE

Maple syrup urine disease results from an autosomal recessive deficiency of branched-chain ketoacid dehydrogenase. Affected children have elevated plasma and urine levels of the branched-chain amino acids leucine, valine, and isoleucine, as well as elevated levels of their corresponding α-ketoacids and α-hydroxyacids. Clinically, the urine has an odor of maple syrup or burnt sugar; if untreated, brain damage and death occur by the end of the first year.

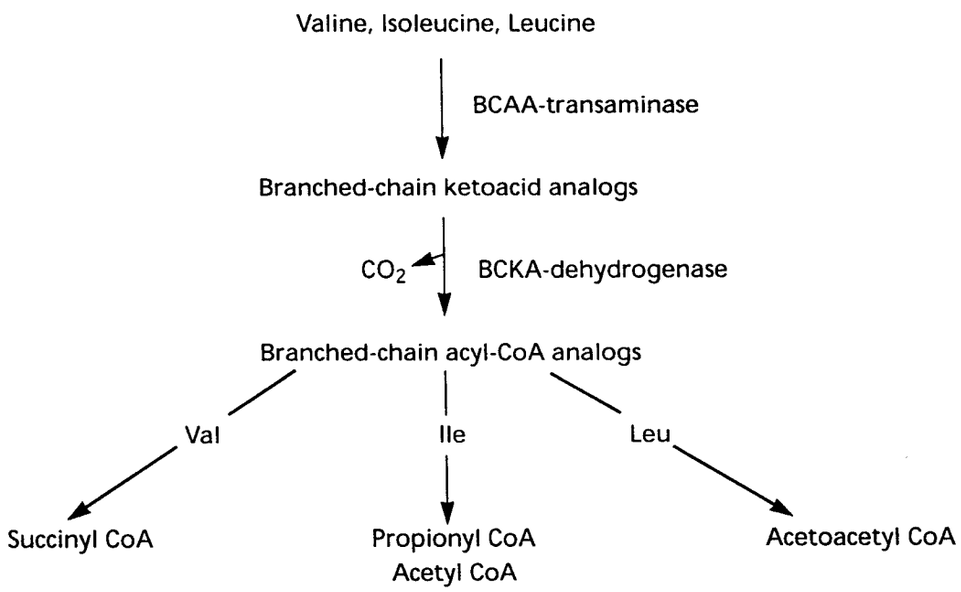


Figure 6-6. Degradation of branched-chain amino acids.

The BCKA-DH is a multienzyme complex structurally homologous to pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. All three catalyze the oxidative decarboxylation of α-ketoacids, and they are composed of three distinct enzymes that require the actions of five coenzymes (NAD^+, FAD, CoA, thiamine-PP, and lipoic acid). They are all located in the mitochondria of most cells.

D. **Assimilation of propionyl-CoA into the TCA cycle.** Propionyl-CoA results from the degradation of isoleucine and methionine, and from the ω-end of odd-chain fatty acids. The pathway for converting propionyl-CoA into succinyl-CoA is shown in Figure 6-7. A deficiency in either propionyl-CoA carboxylase or methylmalonyl-CoA mutase results in organic aciduria. This condition is usually seen in children who present with signs of acidosis.

Chromatographic analysis of blood and urine are an important part of the diagnosis. Treatment protocols include measures to counteract the acidosis, followed by restriction of dietary protein. Individuals excreting excessive amounts of organic acids are at risk for developing a secondary carnitine deficiency, leading to impaired fatty acid oxidation. These secondary effects can be prevented by supplementation with L-carnitine.

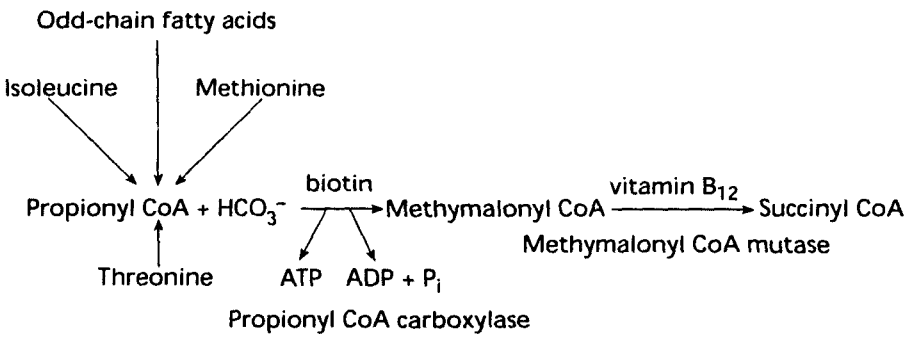


Figure 6-7. Conversion of propionyl-CoA to succinyl-CoA.

1. **Propionic aciduria** results from a deficiency of biotin, propionyl-CoA carboxylase, or the enzyme that covalently attaches biotin to all carboxylases. In the latter case, additional organic acids accumulate.
2. **Methylmalonic aciduria** results from a deficiency in vitamin B_{12} or a defect in methylmalonyl-CoA mutase. Some patients respond favorably to megadoses of vitamin B_{12}.

SYNTHESIS OF NONESSENTIAL AMINO ACIDS

The pathways for amino acid synthesis are complex. All of the nonessential amino acids are synthesized from intermediates in glycolysis or the TCA cycle, from essential amino acids, or from interorgan pathways. Only the key concepts of these pathways will be reviewed.

A. **Synthesis from intermediates in glycolysis or the TCA cycle.** Eight of the nonessential amino acids can be synthesized from intermediates in glycolysis or from the TCA cycle intermediates. This is illustrated in Figure 6-8.

   The transamination of pyruvate, oxaloacetate, and α-ketoglutarate give rise to alanine, aspartate,and glutamate, respectively. The addition of an amide group to the side chains of aspartate and glutamate result in asparagine and glutamine. Phosphoglycerate is a precursor of serine and glycine.

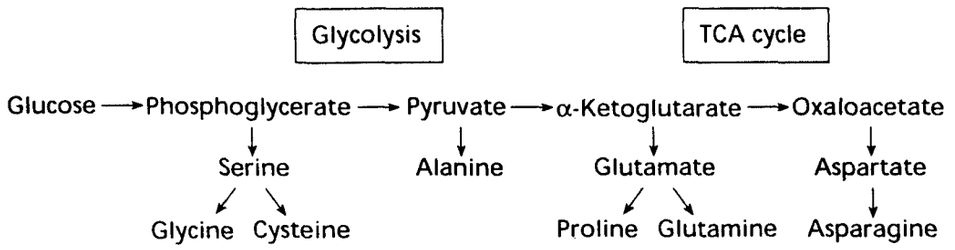


Figure 6-8. Precursors for the nonessential amino acids.

B. **Synthesis from essential amino acids.** Phenylalanine is the precursor for tyrosine, and methionine provides the sulfur for cysteine synthesis. Therefore, if either phenylalanine or methionine is deficient in the diet, tyrosine and cysteine also become essential amino acids. This situation may occur transiently in premature babies. The enzyme systems for synthesizing tyrosine and cysteine are developed late in gestation.

1. **Tyrosine.** The conversion of phenylalanine to tyrosine is catalyzed by **phenylalanine hydroxylase** (Figure 6-9). This enzyme requires O_2 and **tetrahydrobiopterin (THB)**. During the hydroxylation reaction, THB is oxidized to dihydrobiopterin (DHB). For the synthesis of tyrosine to continue, DHB must be reduced back to THB. The regeneration of THB requires NADPH and dihydrobiopterin reductase.

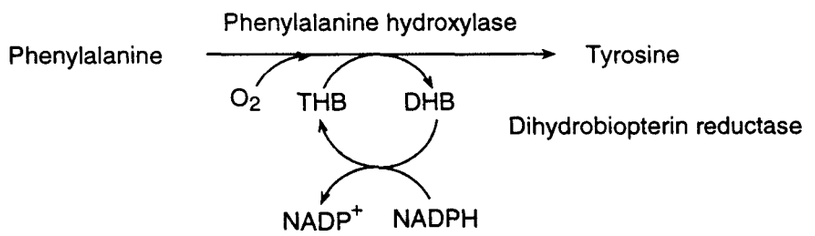


Figure 6-9. Hydroxylation of phenylalanine to tyrosine.

2. **Cysteine.** Serine and methionine are required for the synthesis of cysteine. Key steps in the pathway are shown in Figure 6-10. The sulfur atom in cysteine originates in dietary methionine, and the remainder of the cysteine molecule is derived from serine. Methionine is first converted to homocysteine through several steps. Cystathionine synthase, an enzyme requiring pyridoxal phosphate, condenses homocysteine with serine to form an adduct of the two amino acids. Cystathionase then cleaves the adduct so that the sulfur atom is attached to serine, generating cysteine. Excess homocysteine can be converted back to methionine by the addition of a methyl group from tetrahydrofolate. **Methionine synthase** is the only enzyme in

CLINICAL CORRELATE

Phenylketonuria (PKU) results from either a deficiency of phenylalanine hydroxylase or dihydrobiopterin reductase. There is therefore a buildup of phenylalanine, phenylpyruvate, phenylacetate, and phenyllactate in both plasma and urine, and tyrosine becomes an essential amino acid. Clinically, there is a musty body odor and mental retardation. Treatment is to remove phenylalanine from the diet (including products containing NutraSweet® brand aspartame).

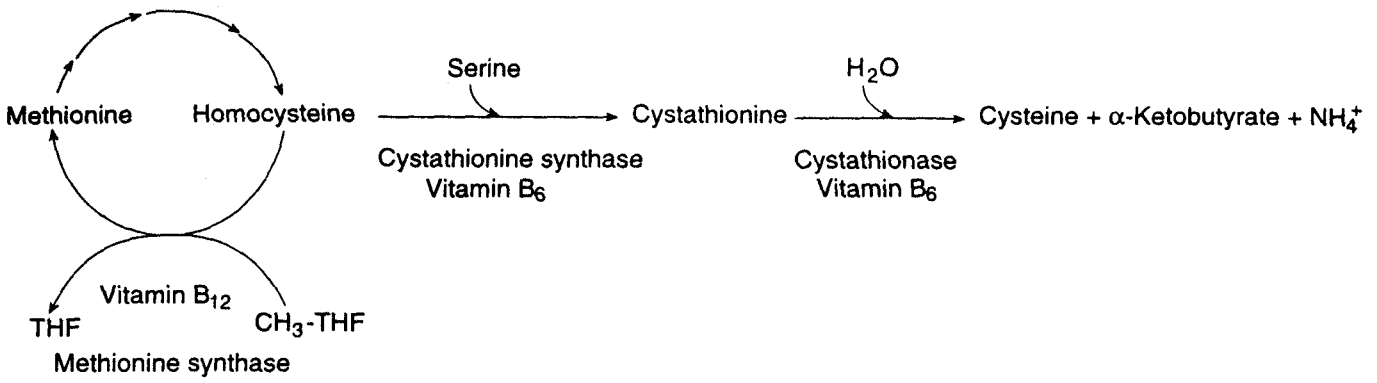


Figure 6-10. Synthesis of cysteine from methionine and serine.

human biochemistry known to use THF as a methylating agent. This is also one of the two reactions in humans that requires vitamin B_{12}.

a. **Homocystinuria** is characterized by excretion of large amounts of homocystine in the urine. It is most frequently seen in children who present with failure to thrive and visual problems due to displacement of the lens. Homocystinuria may be either acquired or inherited. A deficiency in pyridoxine, folate, or vitamin B_{12}, or an inherited defect in either cystathionine synthetase or methionine synthase all result in the accumulation of homocysteine, which is readily oxidized to its disulfide form, homocystine. Cystathionine synthase requires pyridoxal phosphate (the activated form of vitamin B_6) as a cofactor, and methionine synthase requires coenzymes derived form both folate and B_{12}.

b. **Cystathioninuria** results from a deficiency in pyridoxine or from a genetic defect in cystathionase. Large amounts of cystathionine are found in the urine and blood.

C. **Interorgan synthesis of arginine.** The synthesis of arginine in humans is dependent on cooperation between the intestinal mucosa and the kidney. The first two enzymes of the urea cycle are found in intestinal cells, and the third and fourth enzymes of the cycle are found in the kidney. Only liver has all of the enzymes. Intestinal cells synthesize citrulline and release it into the blood. Citrulline is extracted by the kidney and converted to arginine. The kidney has low levels of arginase activity, thus arginine accumulates; however, it does not accumulate in liver due to the high level of arginase activity in the urea cycle.

SPECIALIZED PRODUCTS DERIVED FROM AMINO ACIDS

The synthesis of many of the specialized products, especially the biogenic amines, uses three types of reactions: decarboxylation, hydrox-

ylation, and methylation. Decarboxylation reactions require pyridoxal phosphate; most of the hydroxylations require tetrahydrobiopterin (THB) as a reductant; the methylation reactions require S-adenosylmethionine as a methylating agent. Other specialized products derived from amino acids are melanin, creatine, heme, and nitric oxide.

A. **S-adenosylmethionine (SAM).** Almost all methylation reactions require SAM as a methylating agent. The methyl transfer potential of SAM is much higher than that of methyl-THF. SAM is synthesized by the condensation of methionine and ATP (Figure 6-11). A high-energy bond attaches the sulfur atom to adenosine.

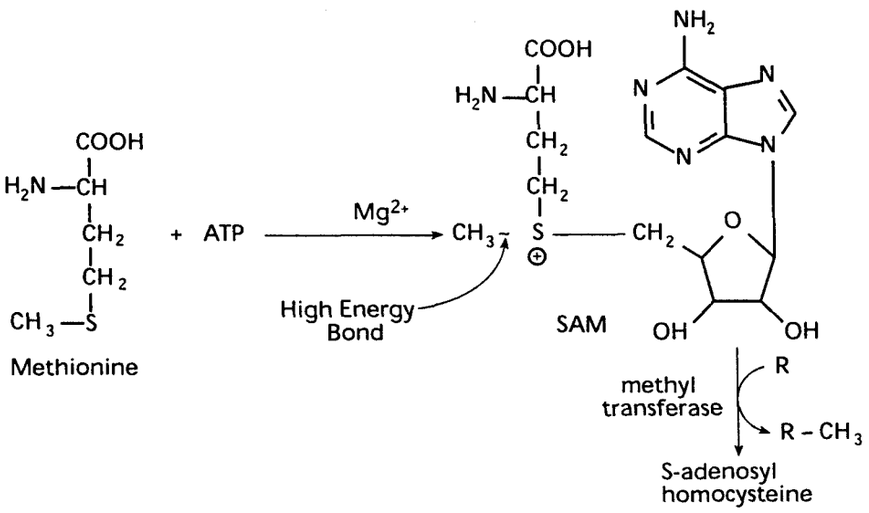


Figure 6-11. Synthesis of S-adenosylmethionine (SAM).

B. **Histamine.** Decarboxylation of histidine results in histamine, an important mediator of inflammatory responses. Histamine is synthesized in mast cells of connective tissue near blood vessels.

$$\text{Histidine} \xrightarrow[\text{Pyridoxal-P}]{\text{Histidine Decarboxylase}} \text{Histamine} + CO_2$$

Figure 6-12. Synthesis of histamine.

C. **γ-Aminobutyric acid (GABA).** Decarboxylation of glutamate results in GABA, an important inhibitory neurotransmitter.

$$\text{Glutamate} \xrightarrow[\text{Pyridoxal-P}]{\text{Glutamate Decarboxylase}} \gamma\text{-Aminobutyric acid (GABA)} + CO_2$$

Figure 6-13. Synthesis of γ-aminobutyric acid.

D. **Serotonin.** Hydroxylation of the aromatic ring of tryptophan followed by its decarboxylation results in serotonin, a potent vasoconstrictor and stimulator of smooth muscle contraction. As shown in Figure 6-14, tryptophan hydroxylase requires O_2 and tetrahydrobiopterin (THB). Dihydrobiopterin reductase and NADPH are required to regenerate THB.

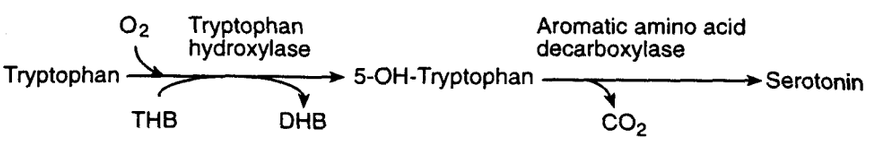


Figure 6-14. Synthesis of serotonin.

CLINICAL CORRELATE

Parkinson's disease results from a decrease of dopamine in the substantia nigra. It is found in approximately 1% of the US population above the age of 55. Symptoms include tremors, postural instability, rigidity, and bradykinesia. The symptoms are treated with L-dopa and carbidopa; the latter selectively inhibits the aromatic acid decarboxylase outside of the central nervous system because it cannot cross the blood-brain barrier.

E. **Catecholamines, melanin,** and **thyroxine** are all derived from tyrosine.

1. **Catecholamines** act as neurotransmitters when synthesized by the brain, and act as hormones when produced by the adrenal medulla. The pathway for synthesis is the same in both tissues (Figure 6-15). It includes hydroxylation, decarboxylation, and methylation reactions. Tyrosine hydroxylase and dopamine-β-hydroxylase have different cofactor requirements.

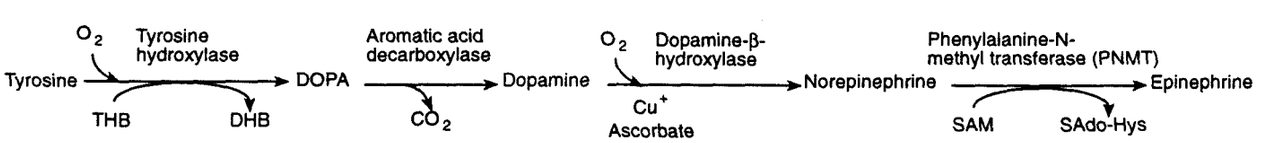


Figure 6-15. Catecholamine synthesis.

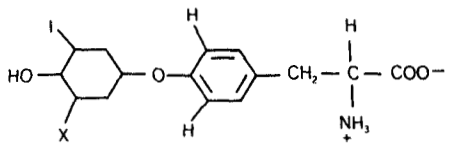
Catecholamines are rapidly degraded by two enzymes that are widely distributed in many tissues: **catechol-O-methyl transferase (COMT)** and **monoamine oxidase (MAO)**.

2. **Melanins** are the pigments of skin and hair that are formed in melanocytes. The initial reaction is catalyzed by **tyrosine hydroxylase**. The isozyme present in melanocytes, also known as tyrosinase, requires copper and ascorbate as cofactors. It hydroxylates tyrosine at two positions, creating a very reactive molecule that polymerizes and gives rise to a series of compounds, collectively known as "melanins." Some forms of **albinism** result from a deficiency in tyrosine hydroxylase.
3. **Thyroxine** synthesis occurs in the follicle cells of the thyroid. Synthesis requires the tyrosine-rich protein, thyroglobulin, and the uptake of iodine. The iodine must be oxidized to I^+

before it can be incorporated into tyrosine side chains. The introduction of one molecule of I^+ gives monoiodonated tyrosine (MIT), while introduction of two molecules of I^+ produces diiodonated tyrosine (DIT). Covalent cross-linking of two DIT molecules results in tetraiodothyronine (T_4), whereas cross-linking of MIT and DIT produces triiodothyronine (T_3). Stimulation by thyroid-stimulating hormone (TSH) results in excision and release of T_3 and T_4.

NOTE

There is only one difference in position between T_3 and T_4:



T_3: X = H
T_4: X = I

F. **Nitric oxide**, also known as "endothelium-derived relaxing factor," is synthesized from arginine in endothelial cells of small blood vessels. Nitric oxide diffuses into the smooth muscle cells, where its direct effect is to stimulate cyclic GMP synthesis. cGMP activates a cascade of events resulting in relaxation of smooth muscle. Nitric oxide is also an important excitatory molecule in the nervous system. It is synthesized from arginine by the enzyme **nitric oxide synthase**. This enzyme requires O_2 and tetrahydrobiopterin as a cofactor. The reaction catalyzed by NO synthase is shown in Figure 6-16. Its synthesis is stimulated by calcium ions. The regeneration of tetrahydrobiopterin requires NADPH.

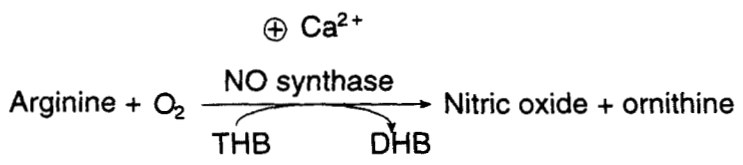


Figure 6-16. Synthesis of nitric oxide.

G. **Creatine synthesis** requires arginine, glycine, and S-adenosylmethionine (Figure 6-17). **Creatine phosphate** is a storage form for high-energy phosphate in muscle. It can be used to rapidly regenerate ATP from ADP by the enzyme creatine phosphokinase (CPK).

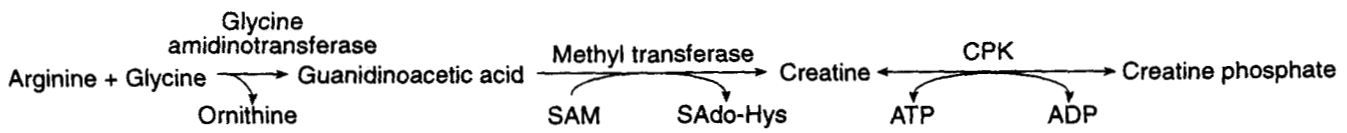


Figure 6-17. Synthesis of creatine and creatine phosphate.

Both creatine and creatine phosphate spontaneously cyclize to form creatinine, which is excreted in urine. Under normal circumstances, the amount of creatinine excreted is constant and is directly proportional to muscle mass. Blood levels of creatinine

are used clinically to assess kidney function, or to estimate muscle mass. The amount of creatinine in a 24-hour urine specimen can also be used to validate the completeness of a 24-hour specimen.

H. **Heme synthesis** requires the assembly of a porphyrin ring, which is derived entirely from glycine and succinyl-CoA (Figure 6-18). The initial step in the synthesis is catalyzed by **Δ-aminolevulinic acid synthase (Δ-ALA synthase)**, an enzyme that requires pyridoxal phosphate. This is the rate-limiting step in heme synthesis and is inhibited by heme, the product of the pathway. Condensation of two mols of Δ-ALA produces a **porphobilinogen**, the first intermediate in the pathway with a pyrrole ring. Condensation of four molecules of porphobilinogen gives a linear pyrrole, which can be cyclized to produce a **porphyrinogen.** Cyclization is catalyzed by uroporphrinogen I synthase, but formation of the isomer that leads to heme requires another protein, uroporphrinogen III cosynthase. The immediate precursor to heme is **protoporphyrin** IX. Insertion of iron by the enzyme heme synthase, also known as ferrochelatase, results in heme.

CLINICAL CORRELATE

Porphyria refers to any abnormality in the pathway of heme synthesis. If the block is early in the pathway, the intermediates that build up are excreted in the urine; if the block is late in the pathway, they are excreted in the urine and feces and accumulate in the skin. Lead poisoning can be considered an acquired porphyria because it inhibits ALA dehydratase and heme synthase (ferrochelatase).

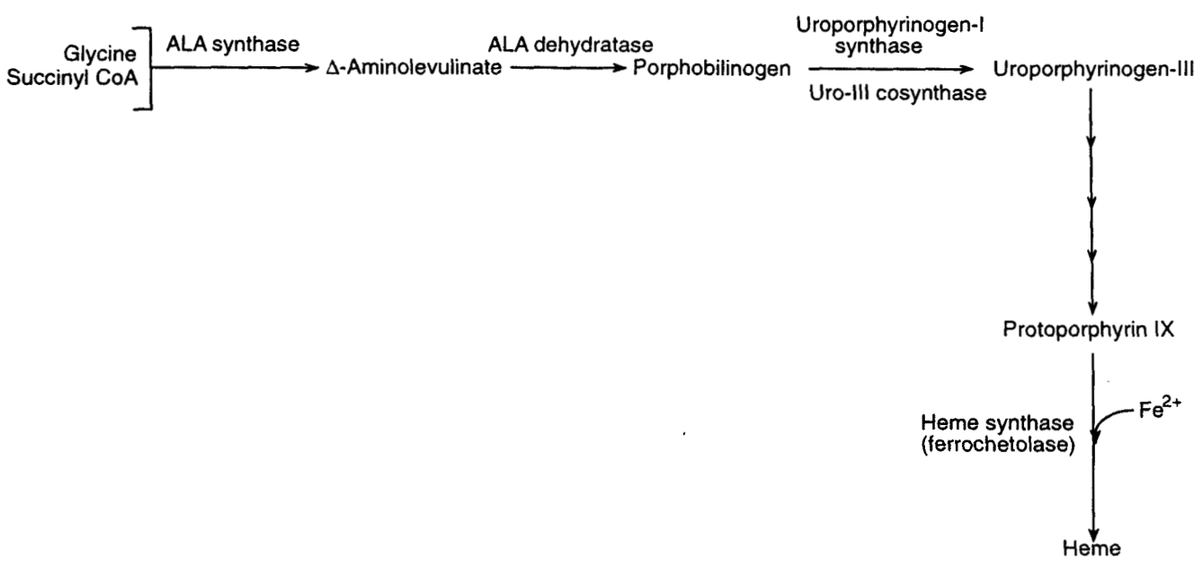


Figure 6-18. Heme synthesis.

CLINICAL CORRELATE

Hyperbilirubinemia can result from massive hemolysis, a block in the catabolism of heme, bile obstruction, or liver damage. Whatever the cause, patients will present with jaundice.

Heme degradation begins in the reticuloendothelial cells, where heme oxygenase opens the ring system to give the linear pyrrole, **biliverdin,** and carbon monoxide (Figure 6-19). This is the only reaction in human biochemistry that generates carbon monoxide. Biliverdin is reduced to **bilirubin,** which is further metabolized in the liver. The addition of either one or two molecules of **glucuronate** to bilirubin greatly increases its solubility. The major

forms of heme that are excreted are **stercobilin** (feces) and **urobilin** (urine). Free bilirubin is known as **"unconjugated"** or **"indirect"** bilirubin, whereas bilirubin glucuronide is known as **"conjugated"** or **"direct"** bilirubin.

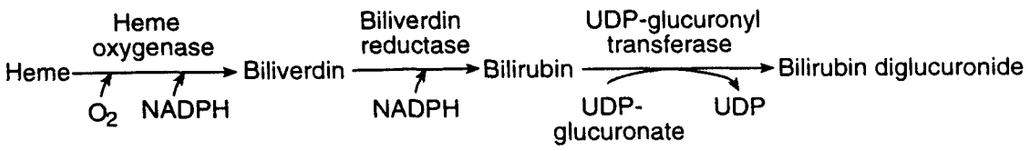


Figure 6-19. Degradation of heme.

Summary of Metabolism

Fuels burned by the body include sugars, fatty acids, ketones, and amino acids. Each fuel has a unique storage form, transport form, and intracellular metabolites. These three forms can be interconverted via separate synthetic and degradative pathways, thus allowing fuel to be stored or mobilized independently. The pathways for storage and mobilization are regulated in a reciprocal fashion, avoiding futile cycles created by simultaneous synthesis and degradation. This chapter will review the major storage forms of energy, the types of fuels used by each organ, the hormones that regulate fuel metabolism, and the transitions that occur in going from a well-fed state to an overnight fast, and finally, to a state of prolonged fasting.

PATHWAYS OF FUEL METABOLISM

Each metabolic fuel has three primary forms: a storage form, a transport form, and unique intracellular intermediates. The relationships among these different forms of each fuel are shown in Figure 7-1.

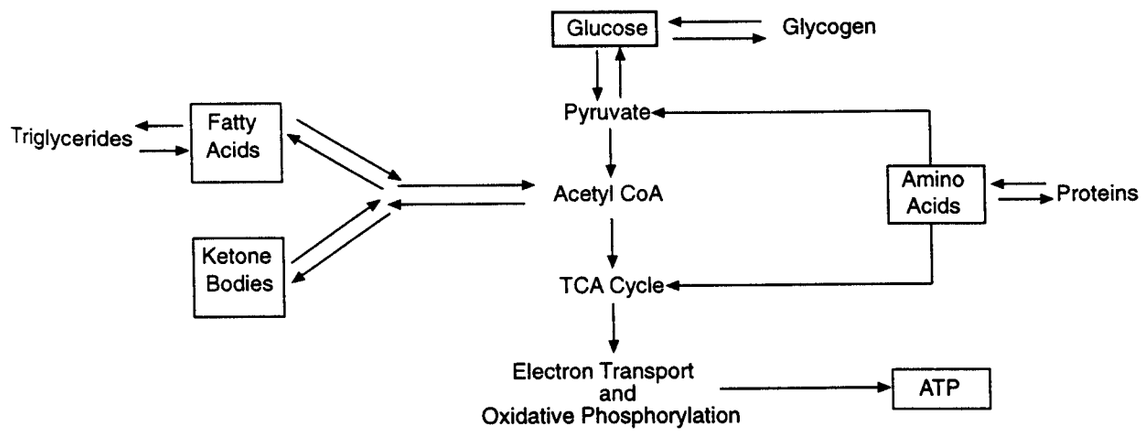


Figure 7-1. Summary of fuel metabolism.

IN A NUTSHELL

Separate pathways for reversible processes prevent the futile cycling of opposing pathways.

A. **Relationship between storage and transport forms.** Fatty ac glucose, and amino acids serve as the transport forms of trig erides, glycogen, and proteins, respectively. The transport fo are found in highest concentration in the blood. They can eit be released or taken up by cells, depending on the nutritio status of the organism. The formation of ketone bodies provid an overflow pathway for excess acetyl-CoA. When fatty acid o dation is producing acetyl-CoA faster than it can be used, t excess acetyl-CoA is converted to ketones. The ketones can th enter the blood and be taken up by other tissues and used fuel. The pathways by which storage forms of fuel are converte to transport forms and vice-versa are unique, and have bee reviewed in detail in other chapters. For example, the incorpora tion of fatty acids into triglyceride and the release of fatty acids from triglyceride occur by different pathways rather than by the reversal of a common pathway. The same relationship exists for glycogen synthesis and degradation and for protein synthesis and degradation. These different and opposing pathways between storage and transport forms of fuel are usually regulated in a reciprocal manner, ensuring that when one pathway is in operation, the other is dormant.

B. **Relationship between transport forms and intracellular metabolites.** Inside the cell, each of the fuels is converted to one or more characteristic intracellular metabolites. For example, amino acids are degraded to glycolytic or TCA intermediates, glucose is degraded to pyruvate, and fatty acids are oxidized to acetyl-CoA (Figure 7-2). Opposing but separate pathways are also present in cells, allowing these fuel-specific intermediates to be converted to their corresponding transport forms and released into the blood. A more detailed metabolic map of these interconversions is shown in Figure 7-3. Once again, the opposing pathways are tightly regulated so that intermediates are not being synthesized and degraded simultaneously. As shown in Figure 7-2, all of the metabolic fuels are eventually degraded to acetyl-CoA, which is oxidized to CO_2 and H_2O by a common pathway. The energy for synthesizing most of the cellular ATP comes from the TCA cycle, the electron-transport chain, and oxidative phosphorylation.

C. **Transitions between metabolic fuels.** Certain transitions among the various fuels are permissible and others are nonpermissible. Both carbohydrates and proteins can be converted to fatty acids. Carbohydrate can be used for the synthesis of the nonessential amino acids and protein. However, acetyl-CoA cannot be converted to pyruvate and, therefore, cannot act as a precursor for carbohydrate or protein synthesis. The reaction catalyzed by pyru-

vate dehydrogenase (pyruvate to acetyl-CoA) is an irreversible reaction, and there is no opposing enzyme in human tissues that will get around this irreversible step. Therefore, the conversion of fat to carbohydrate and protein is considered a nonpermissible transition.

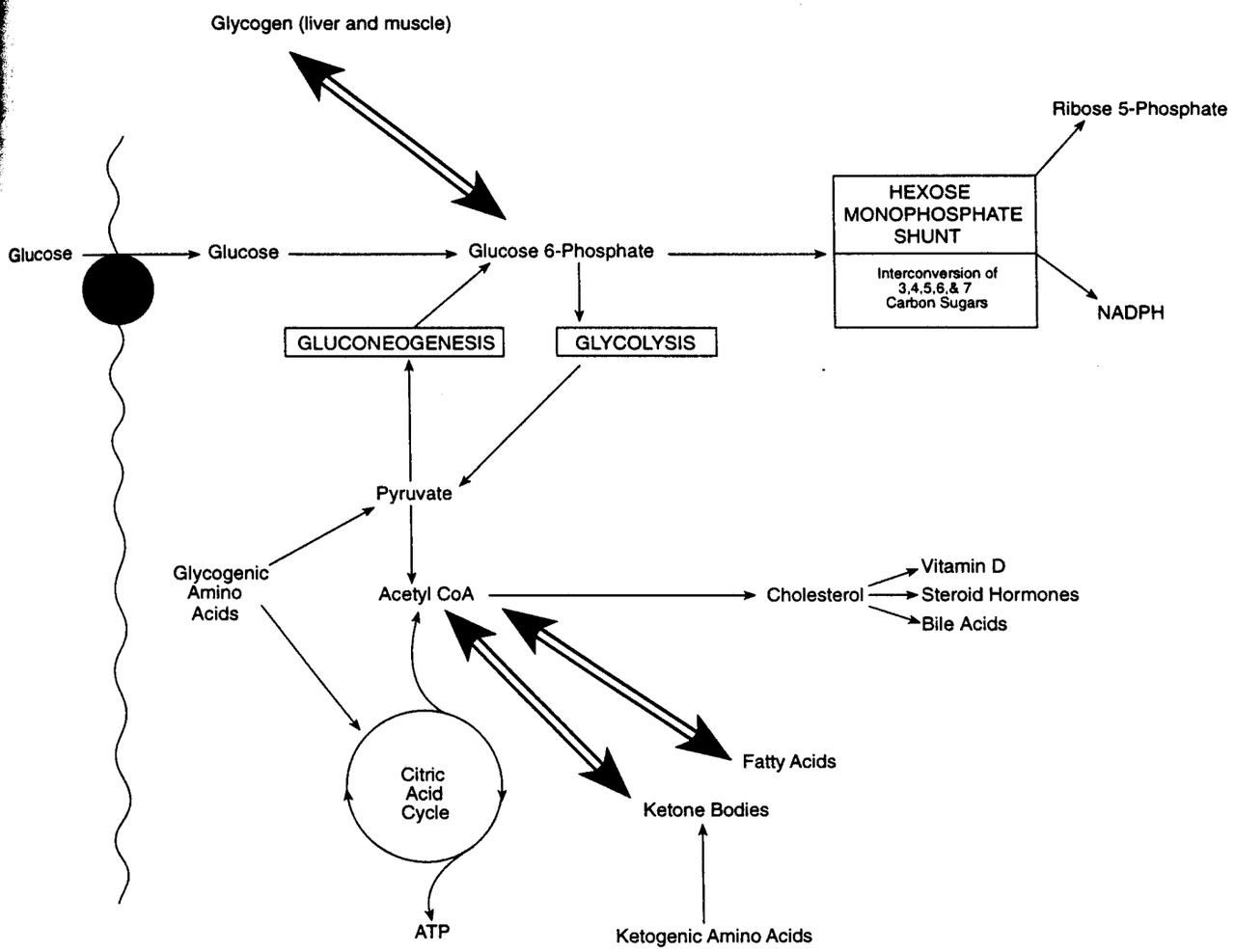


Figure 7-2. Relationship of carbohydrate, fat, and amino acid metabolism.

D. **Regulation of fuel metabolism.** The pathways that are operational in fuel metabolism depend on the nutritional status of the organism. Shifts between storage and mobilization of a particular fuel, as well as shifts between the type of fuel being used, are very pronounced in going from the well-fed state to an overnight fast, and finally to a prolonged state of starvation. The shifting metabolic patterns are regulated mainly by the insulin/glucagon

ratio. Insulin is an anabolic hormone that promotes fuel s
Its action is opposed by a number of hormones, incl
glucagon, epinephrine, cortisol and growth hormone. The
function of glucagon is to respond rapidly to decreased
glucose levels by promoting the synthesis and release of gl
into the circulation.

1. **Well-fed state.** Immediately after a meal, the blood glu
level rises and stimulates the release of insulin. The t
major target tissues for insulin are liver, muscle, and adi
tissue. Insulin promotes glycogen synthesis in liver and mu
After the glycogen stores are filled, liver converts excess g
cose to fatty acids and triglycerides (Figure 7-3). Insulin a
promotes triglyceride synthesis in adipose tissue and protei
synthesis in muscle. Following a meal, most of the energy
needs of the liver are met by the oxidation of excess amino
acids.

NOTE

HMP shunt activity varies with the body's need for reducing power (NADPH). Activity increases during the well-fed state because of the need for NADPH for fatty acid or cholesterol synthesis.

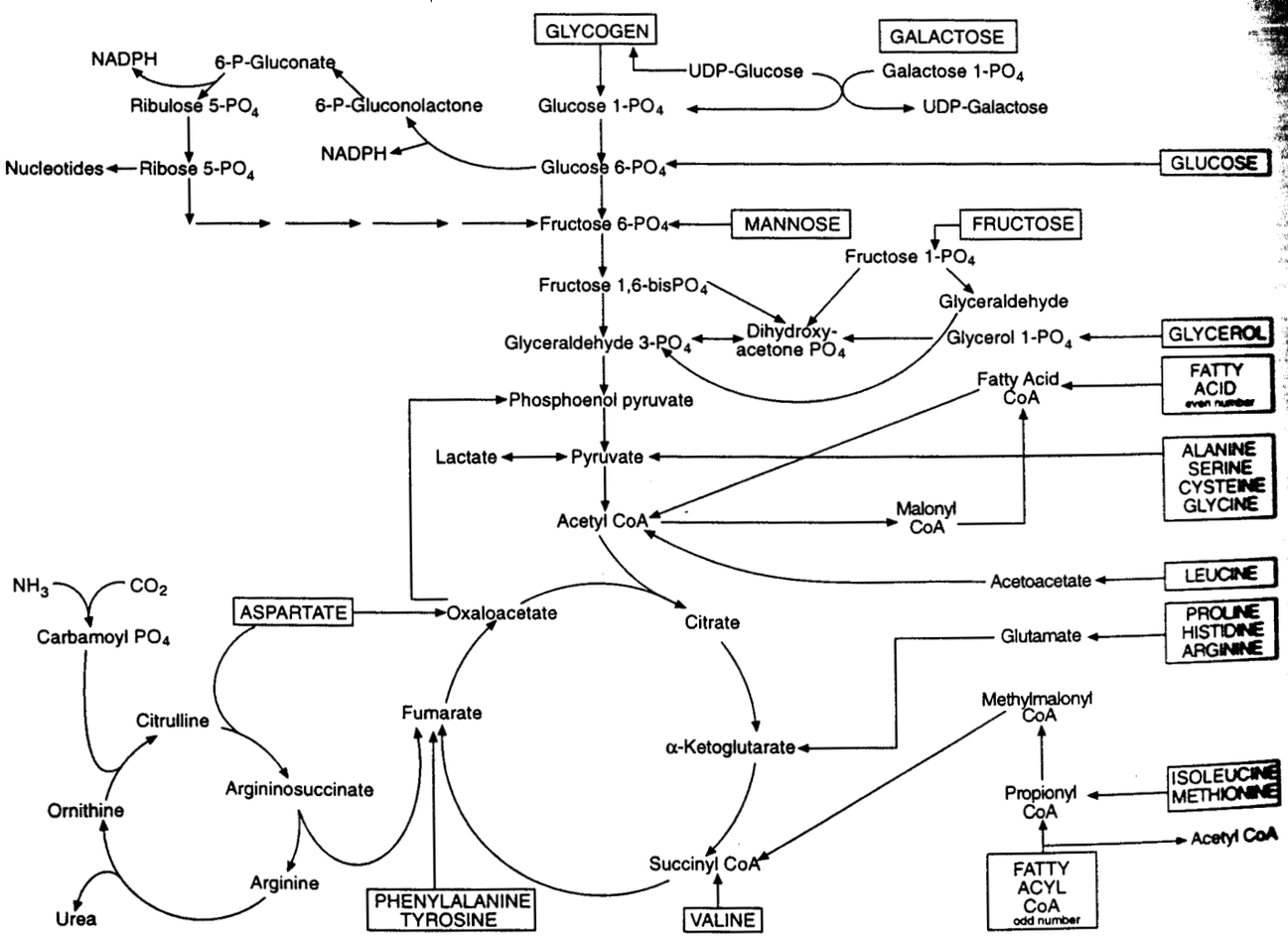


Figure 7-3. Inter-relationships of metabolism.

2. **Overnight fast.** Glucagon and epinephrine levels rise during an overnight fast. These hormones exert their effects on skeletal muscle, adipose tissue, and liver. In liver, glycogen degradation and the release of glucose into blood are stimulated. Hepatic gluconeogenesis is also stimulated by glucagon, but the response is slower than that of glycogenolysis. The release of amino acids from skeletal muscle and fatty acids from adipose tissue are both stimulated by glucagon and epinephrine. The amino acids and fatty acids are taken up by the liver, where the amino acids provide the carbon skeletons and the oxidation of fatty acids provides the ATP necessary for gluconeogenesis.

NOTE

HMP shunt activity decreases with an overnight fast as gluconeogenesis and ketone body formation begin.

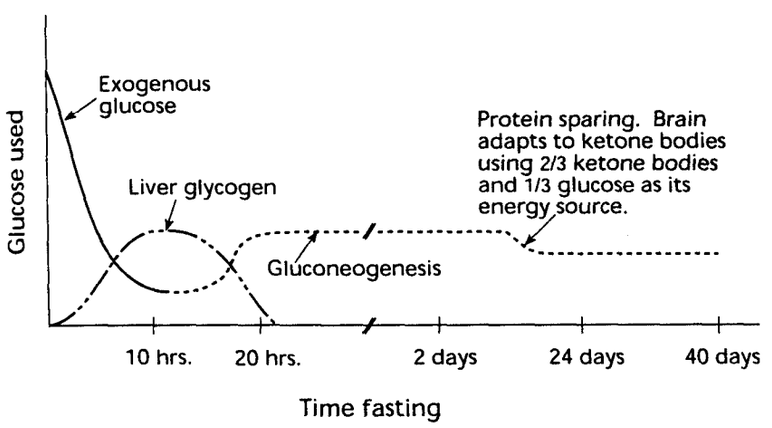


Figure 7-4. Protein sparing effects.

3. **Prolonged fast (starvation).** Levels of glucagon and epinephrine are markedly elevated during starvation. Lipolysis is brisk, resulting in excess acetyl-CoA that is used for ketone synthesis. Levels of both lipids and ketones are therefore increased in the blood. Muscle uses fatty acids as the major fuel and the brain adapts to using ketones for some of its energy. After 1-2 weeks of fasting, the brain derives approximately two-thirds of its energy from ketones and one-third from glucose. The shift from glucose to ketones as the major fuel diminishes the amount of protein that must be degraded to support gluconeogenesis. There is no "energy-storage form" for protein because each protein has a specific function in the cell. Therefore, the shift from using glucose to ketones during starvation spares protein. Red blood cells and renal medullary cells that have few, if any, mitochondria continue to be dependent on glucose for their energy.

NOTE

HMP shunt activity is nonexistent during the prolonged fasting or starvation state.

ENERGY STORAGE AND UTILIZATION

Fats are much more energy-rich than carbohydrates, protein ketones. Complete combustion of fat results in 9 kcal/gram pared to 4 kcal/gram derived from carbohydrate, protein ketones. The storage capacity and pathways for utilization of varies with different organs and with the nutritional status of organism as a whole.

A. **Storage.** Most carbohydrate storage occurs in skeletal muscle liver. Muscle glycogen accounts for approximately two-thirds the carbohydrate storage. A small fraction of the total carbohydrate is found as glucose in the circulation. Fats are stored in pose tissue, which comprises anywhere from one-fourth to on third of the human body weight. Proteins are found in all body tissues where, on the average, they account for three-fourths of the tissue mass. Degradation of more than approximately one-third of the protein in the body is incompatible with life. Therefore, protein sparing by shifting patterns of fuel utilization is essential to survival during prolonged fasting.

B. **Utilization.** Patterns of fuel utilization depend both on the metabolic state (well-fed vs fasting) and on the tissue being studied. Regardless of which fuel is used, they are all degraded to acetyl-CoA, which is oxidized by common pathways to CO_2 and H_2O. The fact that all metabolic fuels give rise to a common intermediate (acetyl-CoA) provides a mechanism for establishing priorities in fuel utilization.

1. **Priorities.** The fasting state promotes fat utilization and inhibits carbohydrate and protein utilization. In the fasting state, gluconeogenesis, proteolysis, and lipolysis are all activated. The amino acids are supplying carbon skeletons for glucose synthesis and the oxidation of fatty acids to acetyl-CoA provides the energy for driving glucose synthesis. The accumulation of acetyl-CoA inhibits pyruvate dehydrogenase, the enzyme that irreversibly converts pyruvate to acetyl-CoA. When pyruvate dehydrogenase is inhibited, the ability to use carbohydrate and protein as sources of fuel is decreased. Under these conditions, pyruvate (derived from either carbohydrate or amino acid precursors) can be diverted away from oxidation and toward gluconeogenesis. Thus, when fat is being used as a metabolic fuel, there is no need to oxidize carbohydrate or protein.
2. **Organ specificity.** Different organs use different fuels. The organ-specific patterns of fuel utilization in the well-fed and fasting states are summarized in Table 7-1.

IN A NUTSHELL

The increase in acetyl-CoA leads to a decrease in the activity of pyruvate dehydrogenase, which in turn leads to a decrease in the utilization of carbohydrates and proteins.

Organ	Post-feeding	Fasting
Skeletal muscle	Glucose	Fatty acids, ketones
Cardiac muscle	Glucose and fatty acids	Fatty acids, ketones
Brain	Glucose	Ketones, glucose
Kidney cortex	Fatty acids	Fatty Acids
Kidney medulla	Glucose	Glucose
Liver	Glucose, amino acids	Glycogen, lactate, glycerol, amino acids, fatty acids
Adipose tissue	Glucose	Fatty acids
Red blood cells	Glucose	Glucose

Table 7-1. Preferred fuels in the well-fed and fasting states.

a. **Skeletal muscle.** The main fuels of skeletal muscle are glucose and free fatty acids. Due to the enormous bulk, skeletal muscle is the body's major consumer of fuel. After a meal, glucose is used to replenish the glycogen stores. In the post-prandial state, elevated levels of insulin promote the uptake of glucose and amino acids by muscle. Excess amino acids are oxidized for energy and excess glucose is broken down through glycolysis. If the muscle is anaerobic due to intense contraction, the production of pyruvate by glycolysis may exceed the capacity of the citric acid cycle to carry out terminal oxidation. In this case, lactic acid accumulates. Pyruvate is converted to lactate so that the NAD^+ needed by glyceraldehyde-3-phosphate can be regenerated. In the fasting state, muscle uses fatty acids and ketones as the primary fuel. Any available glucose is spared for use by brain and red blood cells.

b. **Cardiac muscle.** After a meal, glucose and fatty acids are the major fuel consumed by heart muscle. After a brief period of fasting, fatty acids are used, and after a longer fast, ketones are used. Thus, cardiac muscle behaves much like skeletal muscle in terms of fuel utilization, except it has a much higher oxidative capacity and many more mitochondria.

c. **Brain.** Glucose is the primary fuel for brain. Fatty acids cannot cross the blood-brain barrier and are therefore not used at all. Between meals, brain relies on blood glucose supplied by either hepatic glycogenolysis or gluconeogenesis. Only in prolonged fasts does the brain gain the capacity to use ketones for energy, and even then ketones supply only approximately two-thirds of the fuel supply. The remaining one-third of the fuel continues to be supplied by glucose.

IN A NUTSHELL

- *Glucose and fatty acids are the main fuels in skeletal muscle.*
- *Cardiac muscle uses glucose, fatty acids, and ketone bodies for fuel.*
- *The brain uses glucose for fuel, and will resort to ketone bodies during prolonged fasting only if it's desperate.*

IN A NUTSHELL

The kidney isn't picky: it'll use whatever is available for fuel.

d. **Kidney.** The kidney is unique in that it utilizes virtua
fuels: fatty acids, glucose, lactate, amino acids, c
glycerol, and ketones. The renal cortex relies primar
fatty acids and to a lesser extent on ketones, but wil
use lactate, amino acids, and glycerol. The renal me
preferentially uses glucose. During states of fasting,
rate of gluconeogenesis by the kidney is increased.

e. **Liver.** The two major roles of liver in fuel metabolism are
maintain a constant level of blood glucose under a wi
range of conditions, and to synthesize ketones when ex
fatty acids are being oxidized. After a meal, the gluco
concentration in the portal blood is elevated. The liv
extracts excess glucose and returns the concentration in th
blood to normal. The extracted glucose is used by the liver
to replenish its glycogen stores, and any glucose that
remains is then converted to acetyl-CoA and used for fatty
acid synthesis. The increase in insulin following a meal stim-
ulates both glycogen synthesis and fatty acid synthesis in
liver. The fatty acids are converted to triglycerides and are
released into the blood as VLDLs. In the well-fed state, the
liver derives most of its energy from the oxidation of excess
amino acids. Between meals and during prolonged fasts,
the liver releases glucose into the blood. The increase in
glucagon during fasting promotes both glycogen degrada-
tion and gluconeogenesis. Lactate, glycerol, and amino
acids provide carbon skeletons for glucose synthesis.

f. **Adipose tissue.** After a meal, the elevated insulin stimu-
lates glucose uptake by adipose tissue. Insulin also stimu-
lates fatty acid release from VLDL and chylomicron triglyc-
eride. Lipoprotein lipase, an enzyme found in the capillary
bed of adipose tissue, is activated by insulin. The fatty acids
that are released from lipoproteins are taken up by adipose
tissue and re-esterified to triglyceride for storage. Insulin is
also very effective in suppressing the release of fatty acids
from adipose tissue. During fasting, the increase in
glucagon and epinephrine activates hormone-sensitive
lipase in fat cells, allowing fatty acids to be released into
the circulation.

HORMONAL REGULATION OF METABOLISM

The hormones that regulate fuel metabolism can be divided into two major groups: **anabolic hormones** that promote storage, and **catabolic hormones** that promote mobilization.

A. **Hormones promoting fuel storage.** In humans, insulin is the only hormone that promotes fuel storage. Insulin is produced by the

β-cells in the islets of Langerhans in the pancreas. The release of insulin from the β-cells is stimulated by an increase in the concentration of blood glucose.

B. **Hormones promoting fuel mobilization.** The action of insulin is antagonized by a number of hormones that are collectively referred to as "catabolic hormones." These hormones and their sites of synthesis are listed in Table 7-2. They all increase the rate at which fatty acids, glucose, and amino acids are released from their storage forms.

Hormone	Site of synthesis
Glucagon	Alpha cells of pancreas
Epinephrine	Adrenal medulla
Norepinephrine	Adrenal medulla
Cortisol	Adrenal cortex
Growth hormone[a]	Anterior lobe of pituitary

[a]Growth hormone increases gluconeogenesis in liver and increases liver glycogen.

Table 7-2. Fuel-mobilizing hormones.

C. **Mechanisms of hormone action.** Hormones mediate the transfer of information from one group of cells to another. There are two major types of mechanisms by which informational transfer occurs, depending on where the hormone exerts its effect.

1. **Hormones acting at the plasma membrane of cells.** The binding of hormones by cell surface receptors initiates a chain of events that results in the phosphorylation of specific intracellular proteins. There are several intermediate steps that occur between hormone binding and protein phosphorylation. Most of these hormones generate "second messengers" (cAMP, cGMP, Ca^{2+}, diacylglycerol) inside the cell that stimulate specific protein kinases. An example is shown in Figure 7-5. In this case, the hormone stimulates adenylate cyclase to synthesize cAMP. The synthesis of cAMP requires interaction between three types of proteins: **hormone receptors, G-proteins** that bind GTP or GDP, and **adenylate cyclase.** The G-protein acts as a communication link between the receptor and adenylate cyclase. When the receptor binds hormone, this information is transmitted to adenylate cyclase via the G-protein, and cAMP synthesis begins. (The G protein must bind GTP in order to communicate with adenylate cyclase. The hydrolysis of GTP to GDP will terminate the communication.) The increased cAMP activates a protein kinase that then phosphorylates a few specific enzymes in the cell. The activity of the phosphorylated

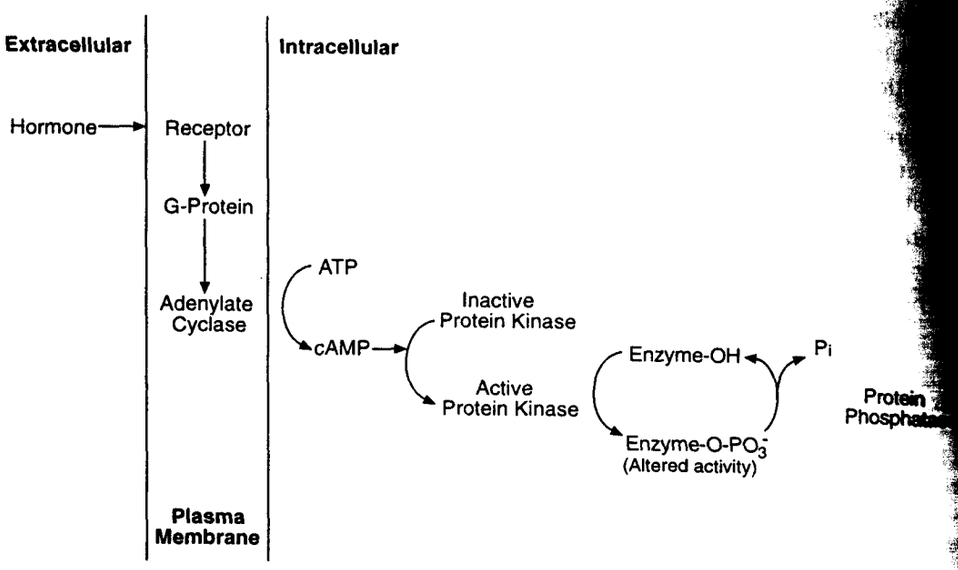


Figure 7-5. Mechanism of peptide hormones that activate adenylate cyclase.

enzyme may be either increased or decreased. The enzyme activity can be restored to its original level by removing the phosphate group, a reaction catalyzed by a family of protein phosphatases.

2. **Hormones that act in the nucleus.** The other major site where hormones exert their effects is in the nucleus of cells, where the hormone-receptor complex binds to DNA and alters the rate of gene expression. Steroid hormones, thyroid hormones, and 1,25-dihydroxycalciferol (activated vitamin D) all bind to specific receptors and alter the rate of mRNA synthesis. Binding of the hormone-receptor complex to "enhancer" sequences in DNA results in increased mRNA synthesis, whereas binding to "silencer" sequences results in decreased mRNA synthesis. The mechanism by which steroid hormones stimulate the transcription of a particular gene is illustrated in Figure 7-6. The enhancer region in the DNA alone has no stimulatory effect on transcription. However, when the hormone-receptor complex binds to the enhancer region, transcription of a specific coding sequence is stimulated.

D. **Mechanisms of specific hormones that regulate fuel metabolism.** The hormones primarily responsible for fuel mobilization are glucagon, catecholamines (epinephrine and norepinephrine), and cortisol. The only hormone that promotes fuel storage is insulin. A brief description of the mechanism for each of these hormones and how they coordinate the various pathways of carbohydrate, fat, and protein metabolism is discussed below.

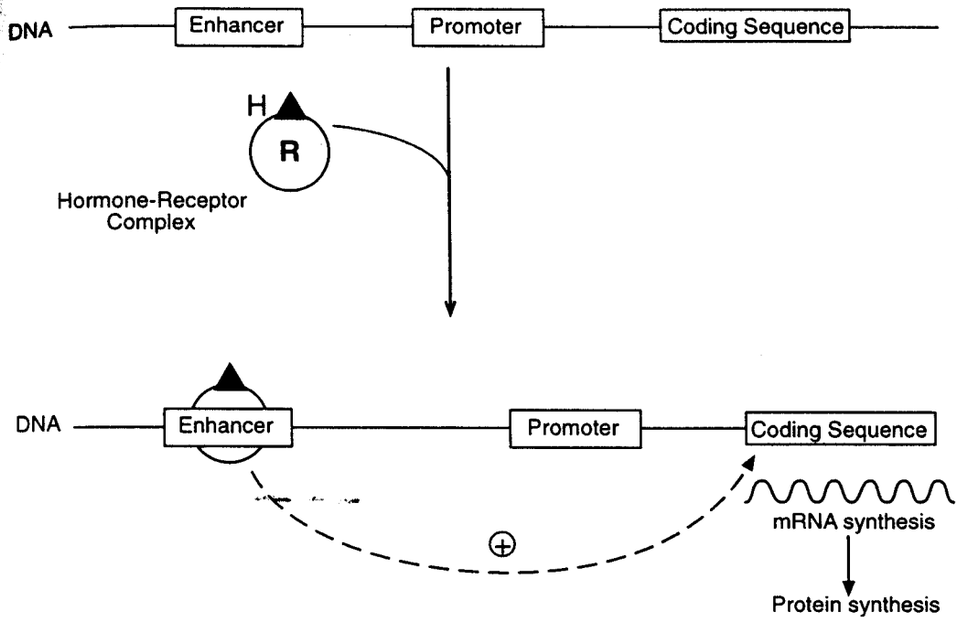


Figure 7-6. Hormonal stimulation of gene expression.

1. **Hormones that activate adenylate cyclase.** Three of the four hormones that mobilize fuel (glucagon, epinephrine, and norepinephrine) bind to cell surface receptors and activate adenylate cyclase, resulting in the synthesis of cAMP and the subsequent effects previously described in Figure 7-5.
   a. **Glucagon** release is stimulated by either low blood glucose, elevated levels of glucogenic amino acids, or by sympathetic stimulation. The primary target for glucagon is the liver, where it stimulates glycogenolysis and gluconeogenesis and inhibits glycolysis. It also activates lipolysis and the release of fatty acids from adipose tissue. The binding of glucagon to receptors in these tissues results in the phosphorylation of a number of enzymes by cAMP-dependent protein kinase. The enzymes that are phosphorylated in response to glucagon are listed in Table 7-3, together with the pathway they participate in and the effect that phosphorylation has on the enzyme.
   b. **Epinephrine and norepinephrine** are released from the adrenal medulla in response to hypoglycemia. These hormones have two major types of receptors, α and β. When the β receptors are occupied, adenylate cyclase is activated and cAMP levels are increased. Glycogenolysis in cardiac and skeletal muscle and lipolysis in adipose tissue are all increased. Binding of epinephrine to α receptors on liver

IN A NUTSHELL

Glucagon, epinephrine, and norepinephrine all increase cAMP.

Enzyme	Pathway	Effect of phosphorylation
Carbohydrate metabolism		
Glycogen phosphorylase	Glycogenolysis	Activation
Glycogen synthase	Glycogenesis	Inhibition
Pyruvate kinase	Glycolysis	Inhibition
Pyruvate dehydrogenase	Links glycolysis with TCA Cycle	Inhibition
Phosphofructokinase-2	Synthesis of F-2,6-P_2[a]	Inhibition
Fructose-2,6-bisphosphatase	Degradation of F-2,6-P_2	Activation
Lipid metabolism		
Hormone-sensitive lipase	Lipolysis	Activation
Acetyl-CoA carboxylase	Fatty acid synthesis	Inhibition
HMG-CoA synthase	Cholesterol synthesis	Inhibited

[a]Fructose-2,6-bisphosphate is both an allosteric activator of glycolysis and an inhibitor of gluconeogenesis.

Table 7-3. Effect of phosphorylation on key enzymes in carbohydrates and lipid metabolism.

cells results in other second messengers (diacylglycerol and calcium) and stimulation of other protein kinases, ultimately resulting in the activation of glycogenolysis and gluconeogenesis. Epinephrine also increases the heart rate and the force of heart contraction, stimulates contraction of bronchial smooth muscle, and increases blood pressure.

2. **Insulin lowers cAMP levels.** Insulin antagonizes the effects of glucagon by mechanisms that are not entirely understood. For example, glucagon promotes phosphorylation of the intracellular proteins described above, and insulin promotes dephosphorylation of these same proteins. In liver, insulin activates cAMP phosphodiesterase, the enzyme that degrades cAMP. As cAMP levels in cells drop, protein phosphatases begin to shift the equilibrium from the phospho- to the dephospho- forms of these enzymes.

$$cAMP + H_2O \xrightarrow[\oplus \text{insulin}]{\text{cAMP phosphodiesterase}} 5'AMP$$

3. **Hormones that modulate gene expression.** Steroid and thyroid hormones exert their effects by altering the rate of mRNA synthesis for specific proteins or sets of proteins. Glucagon and insulin can also alter the rate of mRNA synthesis for key regulatory enzymes in glycolysis and gluconeogenesis (discussed below).

a. **Cortisol** functions mainly to increase *de novo* synthesis of glucose. Cortisol is synthesized and secreted by the adrenal cortex. Its synthesis is stimulated by ACTH, a hormone released from the anterior pituitary in times of stress. Cortisol has three major target tissues: liver, adipose tissue, and skeletal muscle. The enzymes whose synthesis is induced by cortisol are summarized in Table 7-4. Cortisol results in increased lipolysis and proteolysis in adipose tissue and muscle, respectively. The synthesis of glucose from amino acid precursors is increased in liver, as are the glycogen stores. Glucose utilization is decreased in both muscle and adipose tissue.

b. **Thyroid hormones (T_3 and T_4)** also exert their action in the nucleus by altering the rate of gene expression. Some of the metabolic effects of thyroid hormones are increased oxygen consumption, lowering of blood cholesterol, hyperglycemia, and thermogenesis. Receptors for the thyroid hormones are found in virtually all cells of the body, and the interaction of hormone with receptor stimulates numerous pathways of protein, lipid, and carbohydrate metabolism.

c. **Glucagon and insulin** can also alter the rate of gene expression. The mechanism by which glucagon alters gene expression involves cAMP-dependent phosphorylation of nuclear proteins that interact with the DNA. The mechanism for insulin is unclear, although its effects are clearly antagonistic to the effects of glucagon. Glucagon increases mRNA synthesis for enzymes that are specific for gluconeogenesis (PEPCK, FBPase-I, and glucose-6-phosphatase) and represses the synthesis for key glycolytic enzymes (glucokinase, PFK-1, and pyruvate kinase). Insulin stimulates the synthesis of mRNA for the key glycolytic enzymes and represses mRNA synthesis for gluconeogenic enzymes.

Tissue and enzyme	Pathway	Consequence
Liver		
FBPase-1	Gluconeogenesis	Increased glucose release
PEPCK	Gluconeogenesis	Increased glycogen stored Increased glucose release
Adipose tissue		
Hormone-sensitive	Lipolysis	Increased release of fatty acids (fuel for gluconeogenesis)
Skeletal muscle		
Proteases	Protein degradation	Increased amino acid release (substrate for gluconeogenesis)

Table 7-4. Induction of enzyme synthesis by cortisol.

IN A NUTSHELL

Glucagon:

- ↑ *PEPCK*
- ↑ *FBPase-I*
- ↑ *Glucose-6-phosphatase*
- ↓ *Glucokinase*
- ↓ *PFK-1*
- ↓ *Pyruvate kinase*

PART IV

MOLECULAR BIOLOGY

Nucleic Acids

The nucleic acids DNA and RNA are the informational macromolecules of the cell. The building blocks for nucleic acids are nucleotides, which are arranged in a specific linear sequence and are linked together by phosphodiester bonds between the 3'-OH of one sugar and the 5' phosphate ester of the adjacent sugar. All of the information that specifies the properties and functions of a cell is programmed into the nucleotide sequence of DNA. DNA normally exists as a double helix. The helix is stabilized by noncovalent interactions between the purine and pyrimidine bases in opposite strands of the DNA duplex. This chapter will review the chemical structure and the nomenclature of the DNA and RNA building blocks, the types of interactions that stabilize the secondary structure of nucleic acids, the conformations of DNA, and the metabolism of the purine and pyrimidine bases. Disease states associated with purine and pyrimidine metabolism will also be reviewed.

NUCLEIC ACID STRUCTURE

Nucleic acids are polymers of nucleotides covalently linked to one another. The primary structure of nucleic acids refers to the sequence of the nucleotides in DNA or RNA. Higher orders of structure are stabilized by noncovalent interactions between purine and pyrimidine bases.

A. **Chemical structure of nucleotides.** The nucleotides are the building blocks of nucleic acids. As shown in Figure 1-1, nucleotides consist of three components: a nitrogenous base, a pentose, and a phosphate group. The linkage of the nitrogenous base to the pentose generates a nucleoside. The addition of a phosphate

group to the pentose generates a nucleotide. The genet- mation is carried in the sequence of the nitrogenous bas the sugar and phosphate groups form the backbone polynucleotide chain.

1. **Nitrogenous base.** Nucleic acids contain two types of nitr nous bases, purines and pyrimidines (Figure 1-1A).
   a. **Purines** contain two rings, both with nitrogen ato There are two types of purines found in nucleic acids: a nine (A) and guanine (G).
   b. **Pyrimidines** contain a single nitrogen-containing ring. T three types of pyrimidines found in nucleic acids are cyt sine (C), thymine (T), and uracil (U). Thymine is found on in DNA and uracil is found only in RNA.
2. **Pentose sugar.** RNA contains ribose and DNA contains deoxyri- bose. Deoxyribose differs from ribose in that there is a hydro- gen atom instead of a hydroxyl group attached to the carbon-2 (Figure 1-1C). The carbon atoms of the pentoses in the nucle- ic acids are numbered with primes. For example, the pentose in DNA is 2'-deoxyribose.
3. **Phosphate groups** form the linkages between nucleotides in nucleic acids. In Figure 1-1C, the phosphate group is esterified to the 5' carbon of the pentose. In nucleic acids, phosphodi- ester bonds are formed between adjacent nucleotides. There are three types of phosphodiester linkages found in nucleic acids:
   a. **3'-5' phosphodiester linkage** is the most common linkage and forms the backbone of both DNA and RNA molecules.
   b. **2'-5' phosphodiester linkage** is involved in RNA splicing.
   c. **5'-5' phosphodiester linkage** is found in the cap structure at the 5' end of eukaryotic messenger RNA.

IN A NUTSHELL

Nucleotide:

- *Nitrogenous base*
- *Pentose*
- *Phosphate(s)*

Nucleoside:

- *Nitrogenous base*
- *Pentose*

NOTE

Important nucleotide bonds often refer to the pentose carbons:

- *5'-C phosphate*
- *3'-C hydroxyl*
- *2'-C OH on RNA and 2'-C H on DNA*
- *1'-C glycosidic bond to base*

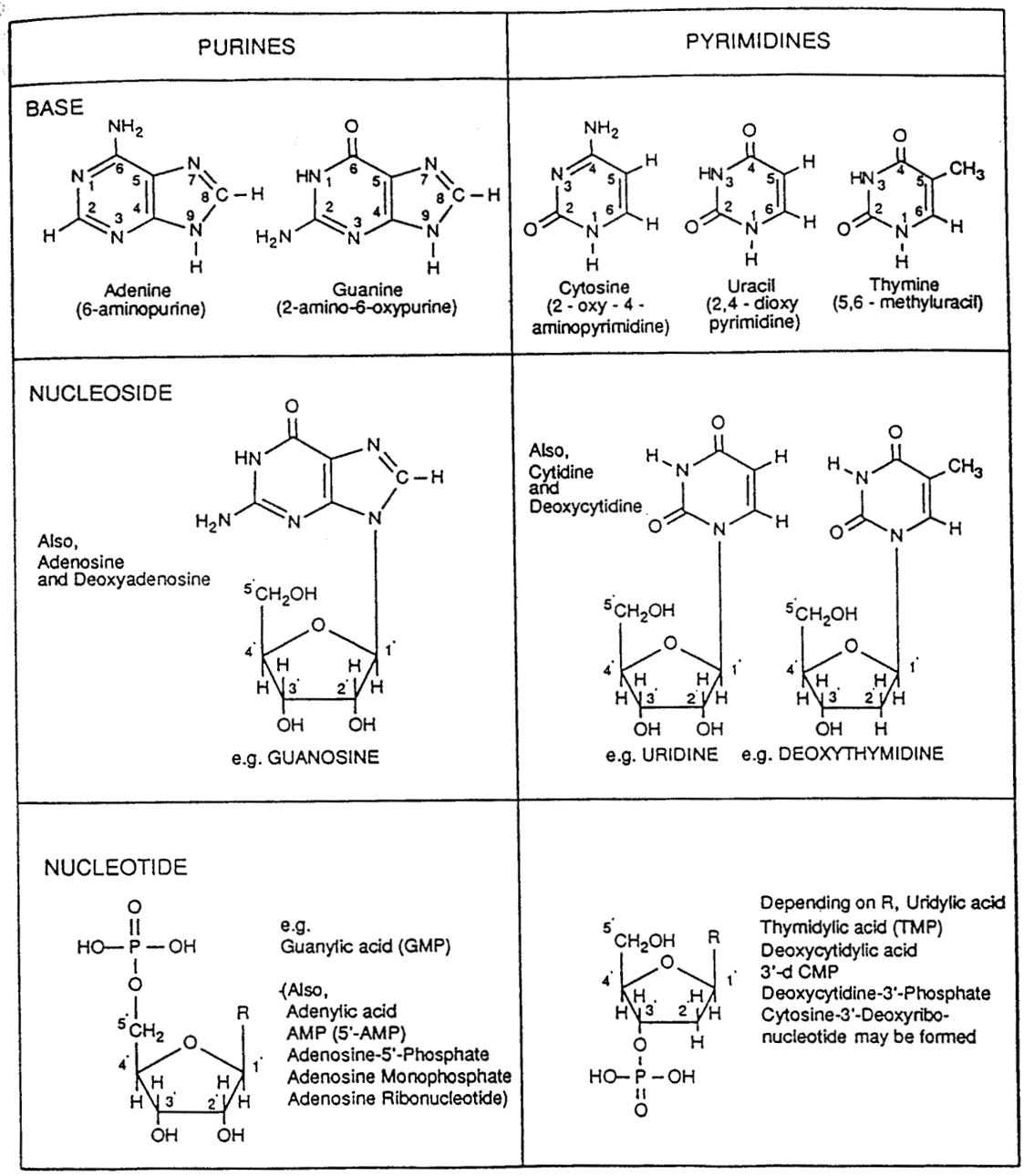


Figure 1-1. Nucleic acid structure.

B. **DNA and RNA are polymers of nucleotides.**

1. **Phosphodiester linkage.** Nucleotides are linked together by phosphodiester bonds formed between the 3′ hydroxyl group on one sugar and the 5′ phosphate group of the adjacent nucleotide (Figure 1-2). The specificity of the nucleotide at a particular position is determined by the complementary base in the template strand.

2. **Polarity**. Polynucleotides have polarity. At one end of polymer, the ribose has a free 3′ hydroxyl group, and the other end the ribose has a 5′ phosphate gro
i.e., 5′ p ——————→ OH 3′

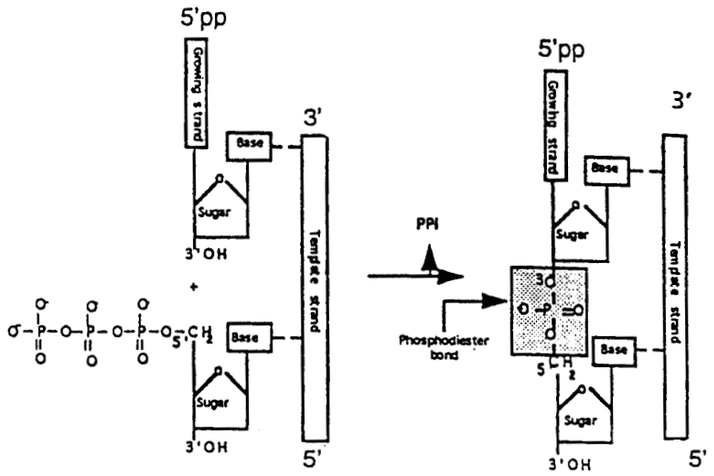


Figure 1-2. Phosphodiester linkage in DNA and RNA.

C. **Major differences in DNA and RNA.** DNA and RNA differ in both structure and function.

1. **Pentose.** DNA contains 2′ deoxyribose, whereas RNA contains ribose.
2. **Nitrogenous bases.** DNA contains A, T, G, and C, whereas RNA contains A, U, G, and C.
3. **Number of strands.** DNA is double-stranded, whereas RNA is single-stranded.
4. **Function.** DNA is the repository of genetic information, whereas RNA represents "working copies" of the DNA that are used in the transfer of genetic information from DNA sequences to protein sequences.

D. **Nomenclature and conventions**

1. **Nomenclature** of nitrogenous bases, nucleosides, and nucleotides. The terminology that distinguishes a free nitrogenous base from a base attached to a pentose (nucleoside), and a base attached to a ribose-5′-phosphate (nucleotide) is summarized in Table 1-1.
2. **Linkage between nitrogenous base and sugar.** In purine nucleosides and nucleotides, the nitrogen-9 of the ring system is linked to the 1′-carbon of the sugar. In pyrimidine nucleosides

IN A NUTSHELL

DNA:

- *H at pentose 2′-C ("deoxy")*
- *Bases are A, T, G, and C*
- *Double-stranded*

RNA:

- *OH at pentose 2′-C ("ribo")*
- *Bases are A, U, G, and C*
- *Single-stranded*

NOTE

Glycosidic bond connects pentose 1′-C to base; phosphodiester bond connects one pentose to another pentose.

Base	(Deoxy) ribonucleoside[a]	(Deoxy) ribonucleotide[a]
Adenine (A)	(Deoxy) adenosine	(Deoxy) adenylate
Guanine (G)	(Deoxy) guanosine	(Deoxy) guanylate
Cytosine (C)	(Deoxy) cytidine	(Deoxy) cytidylate
Thymine (T)[b]	(Deoxy) thymidine	(Deoxy) thymidylate
Uracil (U)[b]	Uridine	Uridylate

[a] Nomenclature for deoxyribonucleosides and deoxyribonucleotides is indicated in parentheses.
[b] Thymine is found only in DNA, whereas uracil is found only in RNA.

Table 1-1. Nomenclature of bases, nucleosides, and nucleotides.

and nucleotides, the nitrogen-1 of the ring is linked to the 1'-carbon of the sugar (see Figure 1-1).

3. **Conventions for writing nucleic acid sequences.** The nucleotide sequence is represented by the single letter codes corresponding to the bases (A, C, G, T, U). Unless otherwise specified, the orientation is written in the direction of synthesis (5' to 3')

ACCTG

If polarity (directionality) is being emphasized, the numbers may be added to the beginning and the end of the letter sequence.

5' ACCTG 3'

If the presence of the phosphate groups is being emphasized, the structure may be written as follows. In the first structure below, there is a free hydroxyl group on the 3' end. In the second structure, there is a phosphate at the 3' end.

5' pApCpCpTpG-OH 3'

5' ApCpCpTpGp 3'

The presence of a 5' triphosphate and a 3' hydroxyl group is indicated by either of the following structures. If a phosphate group is not shown at the 3' end as in the first sequence, it is assumed that there is a free hydroxyl group. In the second structure, the hydroxyl group is drawn in.

5' pppApCpCpTpG 3'

5' pppApCpCpTpG-OH 3'

E. **Secondary structure of nucleic acids.** The secondary structure of DNA refers to its double-stranded duplex structure. Although RNA is almost always a single-stranded molecule, it can bend back on itself to form regions of double-stranded secondary structure.

NOTE

Nucleases cut phosphodiester bonds. Exonucleases remove terminal nucleotides only, while endonucleases cut between internal nucleotides.

IN A NUTSHELL

***1° structure** = nucleotide sequence*

***2° structure** = double helix, which can form between any antiparallel and complementary sequences:*

- *Double-stranded DNA*
- *Inverted complementary sequences within an RNA*
- *RNA and DNA*

1. **Base pairing.** The double-stranded structure is stabilized by base pairing between purines and pyrimidines. Complementary bases that pair with one another are G and C (in both DNA and RNA), A and T (in DNA), and A and U (in RNA).
2. **Antiparallel strands.** As shown in Figure 1-3, the two strands of DNA are always oriented in an antiparallel fashion, where one strand runs in the 3′ to 5′ direction and the other runs in the 5′ to 3′ direction.

Figure 1-3. Double-stranded DNA.

3. **Stabilizing forces.** The two strands are held together by two types of forces, hydrogen bonding and base stacking.
   a. **Hydrogen bonding between base pairs.** A-T and A-U base pairs are stabilized by two hydrogen bonds, and the G-C pair is stabilized by three hydrogen bonds (Figure 1-4). Because of the extra hydrogen bond, G-C base pairs provide more stability to the DNA duplex structure than do A-T or A-U pairs.

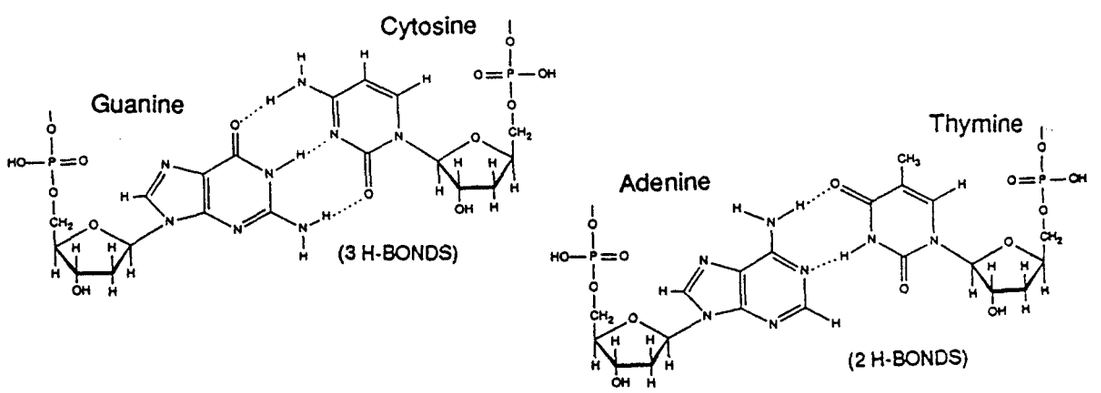


Figure 1-4. Hydrogen bonding between base pairs.

   b. **Base stacking interactions.** The ring structures of the purines and pyrimidines are stacked over one another in the interior of the double helical structure. Hydrophobic

interactions between adjacent purine and pyrimidine rings add stability to the double helix. The base stacking interactions are interrupted by a number of intercalating agents such as actinomycin D, which have been shown to "slide" in between neighboring base pairs in DNA. These agents prevent DNA from serving as an effective template, thus inhibiting replication (DNA → DNA) and transcription (DNA → mRNA).

4. **Destabilization of the double helix.** Double-helical DNA can be denatured by conditions that disrupt hydrogen bonding and hydrophobic interactions, resulting in the "melting" of the double helix into two single strands that separate from one another. No covalent bonds are broken in this process. Some conditions that promote denaturation are:
   a. **Changes in pH.** Addition of sodium hydroxide leads to the ionization of protons associated with the nitrogen atoms in the purine and pyrimidine rings. Ionization disrupts the hydrogen bonds that hold complementary bases together.
   b. **High temperatures.** Temperatures above 80-90°C result in the "melting" of the double helix.
   c. **Chemicals.** Formamide and urea both interfere with hydrogen bonding between bases.

NOTE

Localized "melting" is needed to initiate DNA replication and RNA transcription. DNA sequences needed to initiate DNA replication or RNA transcription are usually A-T rich.

TOPOLOGY OF NUCLEIC ACIDS

Double-stranded DNA may be either linear or circular. Most DNA forms a right-handed, anti-parallel helix.

A. **Conformations of linear DNA.** Linear DNA is the form of double-stranded DNA found in most eukaryotic chromosomes. Three conformations have been identified.

1. **B DNA** is the most common form of DNA. It is the classic Watson-Crick right-handed antiparallel helical structure of DNA in which the bases are stacked upon one another and are perpendicular to the axis of the helix. The helix contains two grooves, a major and a minor groove, that provide sites where DNA-protein interactions occur.
2. **Z DNA** is a rare form of DNA that is found in G-C rich sequences and in DNA sequences that have long tracts of alternating purines and pyrimidines. In contrast to B DNA, this form of DNA forms a left-handed antiparallel helix. The biological function is unknown.
3. **A DNA** is a form of DNA in which the bases are not perfectly perpendicular to the helical axis, but are tilted about 20 degrees away from the axis. This type of DNA is the anhy-

IN A NUTSHELL

- *B DNA is the common, right-handed, antiparallel helical DNA with approximately 10.5 base pairs per helical turn.*
- *Z DNA is a left-handed helical DNA that occurs occasionally within a B DNA chromosome.*

drous form of B DNA. Like B DNA, it is a right-handed antip allel helix. This type of structure is found in RNA-DNA hybrid

B. **Circular DNA has no ends.** This is an advantage because it is re tant to exonucleases. Mitochondrial DNA and the DNA of mo prokaryotes and some viruses are closed circular structure Circular DNA may exist in a relaxed form (no supercoils) or it ma be converted to more compact structures by supercoiling.

1. **Supercoiling.** One of the important properties of closed circu lar DNA is that it can form supercoils, which are important for DNA packaging. The supercoils may be either positive or nega tive.
   a. **Negative supercoiling** twists the DNA in the opposite direction from the clockwise turns of the right-handed double helix. DNA is usually negatively supercoiled, the form required for most biological reactions.
   b. **Positive supercoiling** adds torsional pressure and allows the DNA to be wound more tightly. In positive supercoils, the DNA is twisted in the same direction as the intrinsic winding of the double helix.
2. **Topoisomerases** are enzymes that relieve the tension caused by supercoils in replicating DNA. These enzymes make transient breaks in the DNA by cutting and resealing phosphodiester bonds in the DNA strands.

IN A NUTSHELL

Negative supercoils stabilize partially unwound DNA, promoting replication and transcription. Both circular prokaryotic chromosomes and linear eukaryotic chromosomes contain negative supercoils.

PURINE AND PYRIMIDINE METABOLISM

A. **Synthesis of nucleotides.** Nucleotides are synthesized by either salvage pathways or by the pathway for *de novo* synthesis. In salvage pathways, the nucleosides and the nitrogenous bases that are released by the degradation of nucleic acids are salvaged and reutilized. In the pathway for *de novo* synthesis, the starting materials are more elemental precursors such as amino acids, ammonia, bicarbonate, and ribose-5-phosphate. Tetrahydrofolate is an important coenzyme in purine and pyrimidine synthesis, acting as a donor of C_1 fragments in the *de novo* assembly of the nitrogenous ring systems. In the adult, the salvage pathway is the major route for synthesis and the *de novo* pathway is used primarily by rapidly dividing cells. Deoxyribonucleotides are synthesized by the reduction of ribonucleoside diphosphates. This reaction is catalyzed by ribonucleotide reductase and requires thioredoxin and NADPH as sources of reducing power.

1. **Synthesis of purines.** As shown in Figure 1-5, the purine ring system is synthesized from amino acids, bicarbonate, and C_1 fragments carried by tetrahydrofolate. The purine ring is

NOTE

Deoxynucleotide synthesis inhibitors are used as antineoplastic agents.

- *5 fluorouracil inhibits thymidylate synthetase*
- *Hydroxyurea inhibits ribonucleotide reductase*

These drugs are discussed in the Pharmacology section of the General Principles review book.

attached to ribose throughout the assembly. A parent purine nucleotide, IMP, gives rise to both AMP and GMP. The synthesis of purines is subject to feedback inhibition by GMP and AMP.

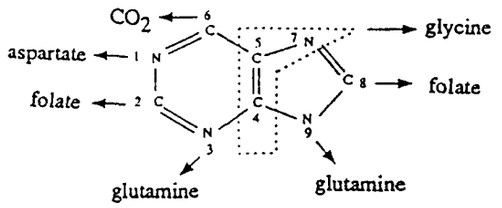


Figure 1-5. Origin of atoms in the purine ring system.

2. **Synthesis of pyrimidines.** In contrast to purines, the pyrimidine ring is completely assembled before it is attached to ribose-5' phosphate. As shown in Figure 1-6, the precursors for the pyrimidine ring are carbamoyl-phosphate and aspartate. The parent nucleotide in the pyrimidine pathway is orotidylate (OMP). Both UTP and CTP are derived from OMP. Thymidine nucleotides (required only for DNA synthesis) are synthesized by methylation of dUMP in a reaction that uses C_1-tetrahydrofolate as a C_1 donor. Pyrimidine biosynthesis is inhibited by CTP and UMP.

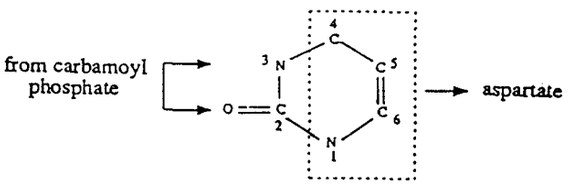


Figure 1-6. Origin of atoms in the pyrimidine ring.

B. **Degradation of nucleotides.** The degradation of nucleotides starts with the removal of the phosphate group and the pentose, yielding free purine and pyrimidine bases. The amino groups attached to the ring system are removed as ammonia by the action of adenosine deaminase and guanine deaminase.

1. **Degradation of purines.** The purine ring system cannot be opened up and degraded. Therefore, the strategy is to make

IN A NUTSHELL

Purines are assembled while attached to ribose-5' phosphate; the pyrimidine ring is completed before it is attached to ribose-5' phosphate.

the ring as soluble as possible so that it can be excreted. The end product of purine degradation is uric acid.

2. **Degradation of pyrimidines.** In contrast to purines, the pyrimidine ring can be opened and partially degraded. Thymidine is degraded to butyrate, whereas uracil and cytosine are degraded to β-alanine.

C. **Disease states** are associated with defects in purine and pyrimidine metabolism. There are a number of clinical conditions that arise from abnormalities in the degradation of purine nucleotides.

1. **Gout** results from the excessive production of uric acid. Elevated levels of uric acid can occur secondary to other diseases that are characterized by high turnover of nucleic acids (i.e., polycythemia and leukemia). In gout, the joints become inflamed due to the precipitation of uric acid crystals. Kidney disease is also seen due to accumulation of uric acid in the tubules. The disease affects mostly males, and is frequently treated with allopurinol, an inhibitor of xanthine oxidase. Xanthine oxidase catalyzes the sequential oxidation of hypoxanthine to xanthine to uric acid.

2. **Lesch-Nyhan syndrome** arises from a deficiency in the enzyme HGPRT (hypoxanthine-guanine phosphoribosyl transferase). This enzyme is a part of the salvage pathway for purines. A deficiency leads to the inability to recycle purines and an overproduction of uric acid. Individuals with Lesch-Nyhan syndrome are mentally retarded, have hyperuricemia, suffer from gout, and have compulsive self-destructive behaviors. The basis for the involvement of the CNS is unknown.

3. **Severe combined immunodeficiency disease (SCID).** A deficiency in adenosine deaminase, an enzyme in the salvage pathway for purines, results in defective development of T and B lymphocytes. Individuals with a deficiency in this enzyme must live in a sterile environment in order to survive. Large amounts of dATP accumulate in red blood cells. This compound allosterically inhibits ribonucleotide reductase, an enzyme essential for DNA synthesis.

The Nucleus, The Cell Cycle, and Meiosis

Most of the cellular events originate in the nucleus. In eukaryotic cells, the nuclear compartment is defined by the nuclear envelope, containing pores that allow macromolecules to pass in and out of the nucleus. The reproduction of cells involves duplication of cellular contents followed by cell division, or mitosis. The process of cell division occupies a small part of the total cell cycle. Most of the cell cycle is spent in interphase, a period of growth and preparation for cell division. Meiosis is a special type of cell division that produces sperm and ova, each of which contains half of the original complement of chromosomes. Each of the gametes produced by meiosis is genetically distinct from one another and from the parent cell. This chapter will review the structure and function of the cell nucleus, the events occurring in interphase and mitosis, and the reductive cell division that occurs during meiosis.

NUCLEUS

The nucleus of the cell serves as a reservoir of genetic information.

A. **Function.** Three cellular functions are carried out in the nucleus.

1. **DNA synthesis** and repair. The process of DNA replication is both rapid and accurate. The nucleus contains all of the enzymes and proteins required for DNA synthesis. Additionally, there are several proofreading and repair mechanisms that correct mistakes that are made spontaneously or by environmental insult.

2. **RNA synthesis.** Transcription of DNA into RNA occurs in the nucleus of eukaryotic cells by three distinct RNA polymerases, each synthesizing a different class of RNA.
3. **RNA processing.** In eukaryotic cells, RNA is synthesized as a precursor that is converted to mature RNA in the nucleus by a number of steps collectively known as "processing."

B. **Structure.** The nucleus is the most conspicuous organelle in eukaryotic cells. It contains almost all of the cellular DNA, and microscopy reveals the following structural features.

1. **Nuclear envelope.** The nucleus is separated from the cytoplasm by a double membrane (inner and outer). The outer membrane is continuous with the rough endoplasmic reticulum and, therefore, is a part of the endomembranous system. The inner and outer membranes form two concentric rings, and are separated by a perinuclear compartment.
2. **Nuclear lamin.** The inner surface of the nuclear envelope is lined by an electron-dense layer containing three major proteins. This network of proteins plays an important role in the structural organization of the nucleus by providing connections between the inner nuclear membrane and the chromatin.
3. **Nuclear pores.** The inner and outer nuclear membranes are fused together at the pores, providing channels of communication between the nucleus and the cytosol. Water, ions, amino acids, and small proteins can freely pass through the pores. The pores are associated with proteins that are required for transport of large macromolecular complexes into and out of the nucleus. The number of nuclear pores is usually fixed in a particular cell type, but may vary at times in relation to the transcriptional activity in the cell.
4. **Chromatin.** The chromosomes and chromatin are composed of DNA that is associated with histones, a family of positively charged proteins.
5. **Nucleolus.** The nucleolus is a very dense region containing large amounts of RNA and functions as the site of ribosome assembly. The nucleolus is associated with the nuclear organizer region (NOR) on the chromosome. The NOR of the DNA contains many copies of the genes for ribosomal RNA. Electron microscopy reveals three distinct zones in the nucleolus. The periphery of the nucleolus has a granular zone that contains ribosomal precursor particles in various stages of assembly. The central region has a fibrillar zone containing ribonuclear protein fibrils. The DNA in the NOR region of the chromosome is a pale-staining region of the nucleolus.

IN A NUTSHELL

Nuclear lamin proteins are important in nuclear disassembly and reassembly during mitosis and meiosis.

CELL CYCLE

The cell cycle begins when a cell comes into existence and ends when the cell divides into two daughter cells. The cell cycle can be divided into two major components, interphase and cell division (Figure 2-1).

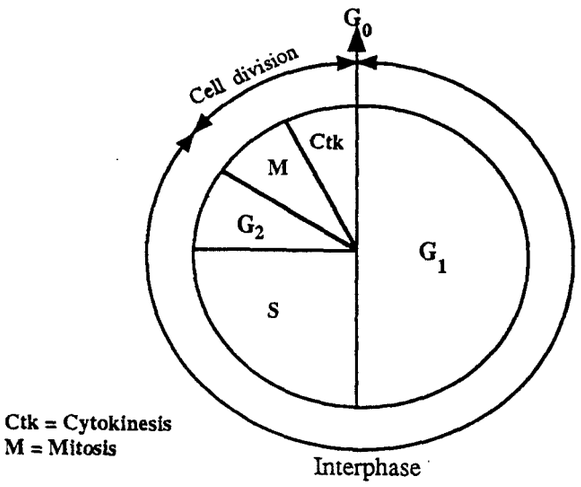


Figure 2-1. The cell cycle.

A. **Interphase** is the period of the cell cycle that precedes mitosis. It can be subdivided into three parts (G_1, S, and G_2) based on discrete events occurring during interphase.

1. **G_1 phase (Gap phase)** is a period of cellular growth that precedes DNA synthesis. In most cells, G_1 lasts approximately 12 hours. During this time, lipids, carbohydrates, and proteins are synthesized. In some cells, G_1 may be very long. For example, in muscle and nerve cells, G_1 is so long that it seems the cells may have completely stopped cycling. Such resting cells are said to be in a special G_1 state called G_0.
2. **S phase (synthesis phase)** usually lasts for 6-8 hours, and it includes the entire period of DNA synthesis and chromosomal replication. The rate of RNA synthesis increases, and the cell prepares for mitosis.
3. **G_2 phase** lasts between 3 and 4 hours, and resembles the G_1 phase in terms of cellular activity, except that the cell is now tetraploid (diploid x 2), whereas in the G_1 phase it was diploid.

B. **Mitosis** is the nuclear division of the cell, resulting in the reduction of the chromosome complement from the tetraploid to the diploid number found in each daughter nucleus. Cytokinesis is the division of the cytoplasm, resulting in the enclosure of each

IN A NUTSHELL

- *Cells perform their differentiated functions while in G_1 (or G_0)*
- *Mitochondria and centrioles, which contain DNA, divide during S phase*
- *During G_2, each chromosome consists of two sister chromatids, connected at the centromere*

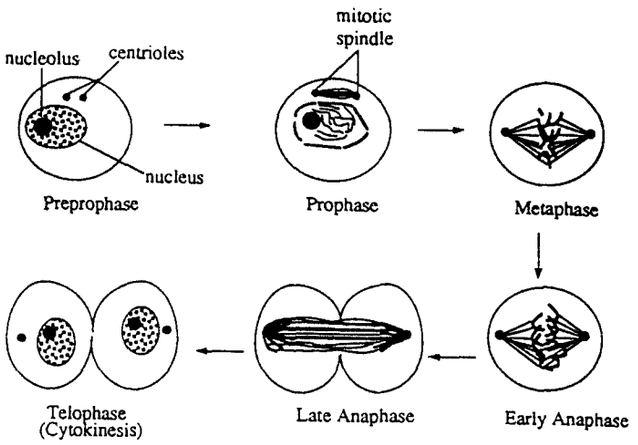


Figure 2-2. Phases of mitosis.

daughter nucleus within a separate cell. As shown in Figure 2-2, mitosis is divided into six phases.

1. **Preprophase.** The chromosomes condense into recognizable thread-like structures in the nucleus. A pair of centrioles (barrel-like structures) are visible in the cytoplasm.
2. **Prophase.** During prophase, the two copies of each chromosome are separated length-wise into two single chromosomes, called chromatids. The main structural component of the mitotic apparatus is the mitotic spindle, which begins to form near the end of prophase. When the centrioles begin to separate, bundles of microtubules assemble between them, creating a spindle. At the end of prophase, the nuclear envelope begins to rupture.
3. **Metaphase.** The nuclear envelope and the nucleolus disappear during metaphase. The spindle moves into the region formerly occupied by the nucleus. The chromosomes move toward the midpoint of the spindle, and each chromosome attaches to bundles of spindle microtubules.
4. **Early anaphase.** The chromatids begin to split longitudinally and start migrating toward the poles of the cell. Once the chromatids split, they are referred to as chromosomes.
5. **Late anaphase.** The chromosomes aggregate at the poles, and a cleavage furrow begins to form, marking the beginning of cytokinesis.
6. **Telophase.** A nucleolus forms and nuclear envelopes appear around each group of daughter chromosomes. The condensed chromatin expands again and begins to reappear. The cytoplasm divides by deepening the cleavage furrow until it forms two daughter cells, thus completing cytokinesis.

IN A NUTSHELL

Until anaphase, each chromosome contains two sister chromatids. After anaphase, each chromatid is now a separate chromosome.

MEIOSIS

Meiosis is the type of cell division that occurs in sperm and ova and reduces the chromosome number by half. It occurs before maturation of the gametes. Genetic recombination also occurs in meiosis, and involves the exchange of segments of chromosomes, resulting in a mixing of allelic linkages into new combinations. This is one of the ways in which deleterious mutations are introduced into the chromosome. As shown in Table 2-1, meiosis involves two complete cell divisions, each resembling the mitotic division previously described. There is no DNA replication during either of the two meiotic divisions. DNA replication occurs before the first meiotic division.

Meiotic division I	Meiotic division II
Prophase I	Prophase II
Metaphase I	Metaphase II
Anaphase I	Anaphase II
Telophase I	Telophase II

Table 2-1. Summary of the stages in meiosis.

A. **First meiotic division.** The six stages of the first meiotic division are illustrated in Figure 2-3 (steps A-F).

1. **Meiotic prophase** occurs in three stages: A, B, and C.
   a. **Stage A.** The chromosomes condense into visible threads, and pairing of homologous chromosomes occurs. The pairing is precise, except for the X-Y chromosome combination in males. The centromeres of homologous chromosomes do not pair.
   b. **Stage B.** Homologous chromosome pairing is complete, and four chromatids appear in each pair; this is a tetrad.
   c. **Stage C.** This is the **recombination** or **cross-over** stage where the interchange of chromatid segments between two paired homologous chromosomes can occur. The point of exchange has an X-like appearance and is called a chiasma. At the chiasma, the chromosome pairs are held together, and at this time large segments of genes are exchanged between homologous chromosomes.
2. **Meiotic metaphase I (stage D).** Paired chromosomes line up on the mitotic spindle. The two chromosomes of each homologous pair form connections with microtubules leading to opposite ends of the cell.
3. **Meiotic anaphase I (stage E).** Both chromatids migrate toward the same end of the cell.

NOTE

- *Cells entering meiosis I are called primary gametocytes (spermatocytes or oocytes); they have the same DNA content as a cell entering mitosis.*
- *Cells entering meiosis II are called secondary gametocytes; they contain 23 chromosomes, each consisting of two sister chromatids.*

NOTE

Recombination occurs between the chromatids within a tetrad; it changes allelic linkages, but not gene sequence.

4. **Meiotic telophase I (stage F).** Each daughter cell gets one member of each chromosome pair, for a total of 23 double-stranded chromosomes.

B. **Second meiotic division.** This is shown in Figure 2-3, step G, and the steps are similar to those in a mitotic division, except that no DNA synthesis occurs prior to this division. The 23 chromosomes divide at the centromere and each of the newly formed daughter germ cells receives 23 chromatids. Therefore, each germ cell now has a haploid number of chromosomes and half the amount of DNA of a diploid somatic cell.

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During meiosis II, sister chromatids of the 23 chromosomes separate. The final cells resulting from meiosis II are spermatids and ova (plus polar body).

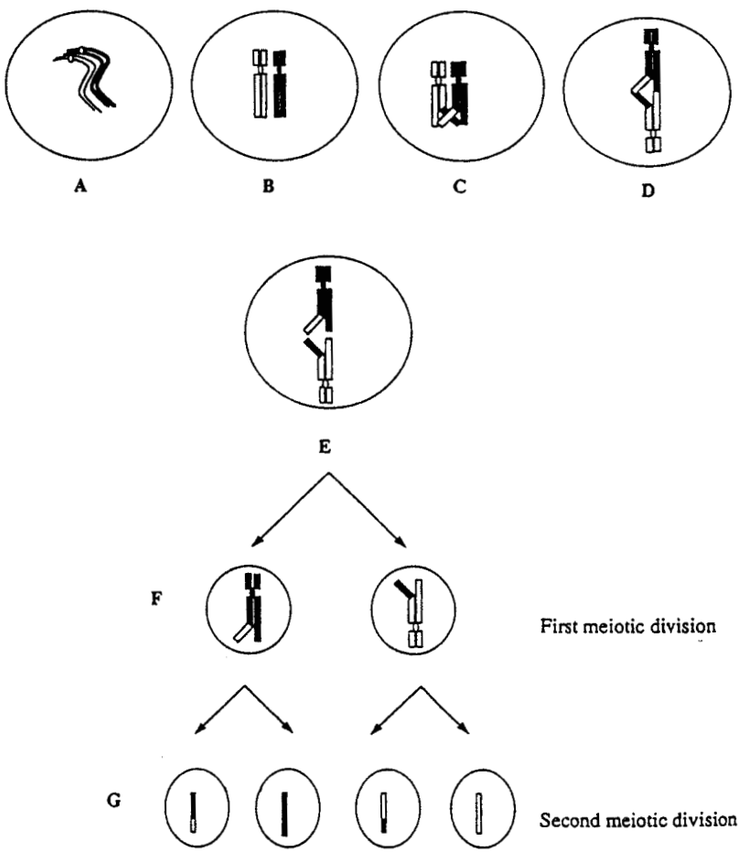

Figure 2-3. First and second meiotic divisions.

DNA Replication and Repair

Genetic information is transmitted from parent to progeny by replication of parental DNA. The process of replication requires a number of proteins; some participate as enzymes in the polymerization of nucleotide; others are responsible for unwinding the double-stranded DNA helix, holding it apart at the point of replication and preventing it from tangling. Maintaining the stability of the genome depends on a number of repair systems that correct occasional mistakes made during replication or that occur after replication due to environmental insult. This chapter reviews the basic concepts underlying DNA replication, the proteins and enzymes involved in replication, and some of the mechanisms that have evolved for repairing DNA. Inhibitors of DNA replication used as chemotherapeutic agents are reviewed, and some clinical abnormalities associated with DNA repair are also discussed.

DNA REPLICATION

Most of the detailed information about DNA replication has been obtained from *in vitro* studies with *E. coli* DNA. Although DNA replication in eukaryotic systems is more complex, the same basic concepts and mechanisms are involved.

A. **General concepts.** The purpose of DNA replication is to create two daughter DNA molecules that are identical to the parental DNA. This is achieved by semi-conservative replication (Figure 3-1).

   1. **Semi-conservative replication** uses each of the parental strands of DNA as a template for directing the synthesis of one new daughter DNA molecule.

2. **Base-pairing** is the process by which each nucleotide in the parental stand is recognized and the complementary base is incorporated in the newly synthesized strand.

   As shown in Figure 3-1, adenine (A) always base pairs with thymine (T), and guanine (G) always base pairs with cytosine (C).

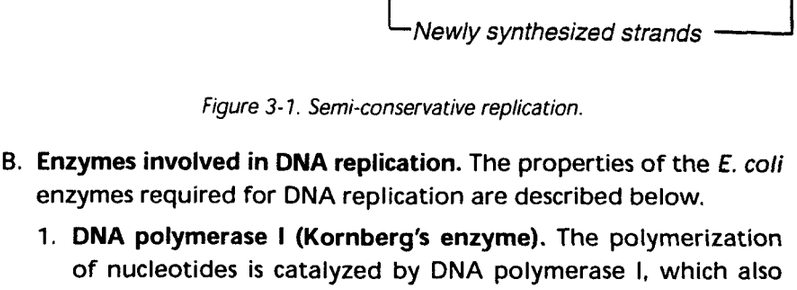

Figure 3-1. Semi-conservative replication.

B. **Enzymes involved in DNA replication.** The properties of the *E. coli* enzymes required for DNA replication are described below.

1. **DNA polymerase I (Kornberg's enzyme).** The polymerization of nucleotides is catalyzed by DNA polymerase I, which also has exonuclease activities that function in proofreading and repair mechanisms.
   a. **Requirements for polymerization.** DNA polymerase I requires all four deoxyribonucleotides (dATP, dTTP, dCTP and dGTP) and Mg^{2+} for activity. Additional requirements are a DNA template (either single- or double-stranded DNA), and a primer with a free 3'-OH group that can react with the first nucleotide. The primer is subsequently erased and the gaps are filled in by the polymerase. Chain elongation proceeds in the direction of 5' to 3'.
   b. **Proofreading function** (3' to 5' exonuclease activity). Occasionally polymerases add a nucleotide to the 3'-OH end that cannot hydrogen-bond to the corresponding base in the template strand. Correct hydrogen bonding is required for polymerization to continue. Thus the polymerase stops and removes the unpaired base from the 3' end so that polymerization can begin again.
   c. **Repair function** (5' to 3' exonuclease activity). The main function of the 5' to 3' exonuclease activity is to erase the nucleotides in the primer.

2. **DNA polymerase II and III.** There are two additional DNA polymerases in *E. coli*. The properties of DNA polymerase III are similar to those of DNA polymerase I. DNA polymerase III is a part of a multiprotein complex and is the major replicating

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All DNA polymerases (prokaryotic, eukaryotic, and viral):

- *Must synthesize 5' to 3'*
- *Must use an antiparallel template*
- *Require a nucleotide (or short nucleic acid sequence) with a free 3'-OH as a primer*
- *Are associated with proofreading enzymes*

enzyme in *E. coli*. The function of DNA polymerase II is unknown.

3. **DNA ligase.** This enzyme catalyzes the formation of a phosphodiester bond between the 3'-OH of one fragment of DNA and the 5'-monophosphate group of an adjacent DNA fragment. These fragments are synthesized as a discontinuous strand in the DNA duplex. The formation of the phosphodiester bond is an endergonic reaction and requires the input of energy that is supplied by NAD^+ in bacteria and by ATP in animal cells and viruses.
4. **Primase.** The primer molecule that is required by DNA polymerase is a short strand of RNA (4-10 bases) that is complementary to the template strands of DNA. The primer is synthesized by a specific RNA polymerase known as primase. The growing end of the RNA primer is a free 3'-OH group. The primase does not require a primer for initiation of polynucleotide synthesis.
5. **Helicases.** Unwinding of the two strands in the DNA helix must occur before DNA replication can begin. This process requires energy (ATP) and enzymes known as helicases that force the two parental strands of DNA to separate at the replication fork.
6. **Single-strand DNA binding (SSB) proteins.** The single strands of DNA resulting from the action of helicase are prevented from reannealing by binding to SSB proteins. These proteins also protect the single-stranded DNA from cleavage by nucleases.
7. **DNA topoisomerases.** These enzymes produce a number of "swivel" points in the DNA molecule that relieve the strain induced by the replication fork. These enzymes cut and reseal the DNA. There are two major classes of topoisomerases, class I and II.
   a. **Class I topoisomerases** relax superhelical DNA in front of the replication fork by creating a transient nick in one of the strands. Class I topoisomerases do not require ATP.
   b. **Class II topoisomerases** introduce negative supercoils in the DNA molecule. This process requires ATP and involves transient breaking and resealing of both strands of DNA.
   c. **Gyrase** is the class II topoisomerase used by *E. coli* to promote DNA helix unwinding during replication.

C. **Events occurring at the replication fork.** As the two strands of parental DNA unwind, a fork-type structure is formed that is referred to as the "replication fork" (Figure 3-2). As DNA synthesis proceeds along the DNA duplex, the replication fork continues

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In both prokaryotes and eukaryotes, each replication fork requires:

- *Primase*
- *DNA polymerases*
- *Helicase*
- *SSBs*
- *Ligase*

to move. The synthesis of one of the daughter strands of DNA occurs continuously (the leading strand) while the other strand (the lagging strand) is made in short fragments (Okazaki fragments) that are then joined together.

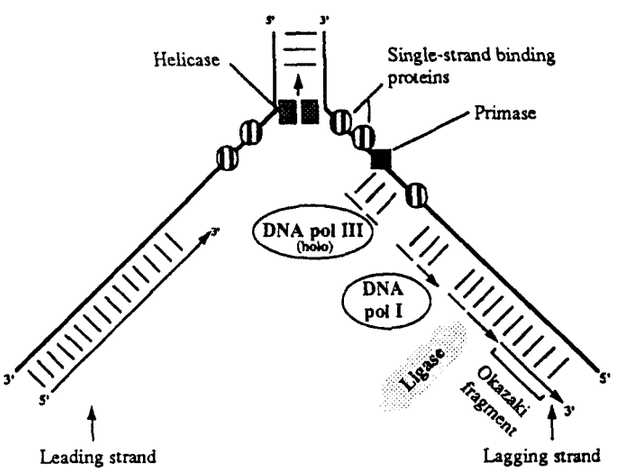

Figure 3-2. Events at the replication fork.

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Both prokaryotic and eukaryotic DNA synthesis is bidirectional:

- *DNA replication begins at an "ori" sequence in the DNA*
- *Two replication forks form at each ori site and move in opposite directions*
- *E. coli DNA contains a single ori site, while eukaryotic chromosomes each contain many ori sites.*

1. **Unwinding of parental DNA.** At the site of replication, the two parental strands do not completely separate, but are unwound in a localized region known as the replication fork. The replication fork can move in either direction from the origin of replication (ori). Unwinding of the helix at the replication fork is accomplished by helicase, and the separation of the single strands is maintained by the binding of several molecules of single-strand binding proteins.
2. **Synthesis of leading strand.** At the replication fork, one strand is synthesized continuously in the 5′ to 3′ direction as the parental DNA duplex molecule unwinds. Thus the leading strand is complementary to the parental strand that runs in the 3′ to 5′ direction.
3. **Synthesis of the lagging strand.** The lagging strand is also synthesized in the 5′ to 3′ direction, but the synthesis occurs discontinuously, producing small fragments (approximately 1000-2000 bases) known as Okazaki fragments. These fragments are later joined by DNA ligase to form a continuous lagging strand.
4. **RNA primers.** There is no known DNA polymerase that can initiate the polymerization of a deoxyribonucleotide chain. Thus, the synthesis of both the leading strand and each of the

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In both prokaryotes and eukaryotes, DNA replication is semi-discontinuous:

- *The leading strand is made continuously*
- *The lagging strand is made in discontinuous Okazaki fragments and then joined*

Okazaki fragments in the lagging strand starts with a short RNA primer (approximately 10 bases). The primer is complementary and antiparallel to the 5′ to 3′ parental strand. The RNA primers are synthesized by a protein complex containing both single-strand binding proteins and primase. This complex binds near the replication fork and moves along the lagging strand as it is opened up by helicase. The 3′ OH of the last ribonucleotide in the primer serves as the site at which the first deoxyribonucleotide is added by DNA polymerase. Elongation of the DNA fragment occurs until it approaches another RNA primer.

5. **Conversion of Okazaki fragments to a continuous strand.** The precursor fragments are eventually joined to form a continuous strand that contains no RNA. This is accomplished by two sequential reactions. When DNA polymerase III approaches another RNA primer, it dissociates and DNA polymerase I enters. DNA polymerase I removes (erases) the ribonucleotides one at a time from the 5′ end (5′ to 3′ exonuclease activity), then adds complementary deoxyribonucleotides to fill in the gaps. Finally, DNA ligase forms a phosphodiester bond between two adjacent DNA fragments.

D. **Inhibitors of DNA replication.** The synthesis of DNA is inhibited by a number of compounds, most of which are either analogs of purines, pyrimidines, or folic acid. These compounds are frequently used as chemotherapeutic agents because of their ability to decrease the rate of division of rapidly growing cancer cells.

1. **Purine analogs.** The most commonly used purine analog inhibitors are 6-mercaptopurine, 8-azaguanine, and 6-thioguanine.
2. **Pyrimidine analogs.** Analogs such as 5-fluorodeoxyuridine and 5-bromodeoxyuridine inhibit thymidylate synthase, an enzyme critical for DNA synthesis. Cytosine arabinoside is an analog of cytosine nucleosides in which the sugar moiety is altered. Insertion of this analog into a DNA chain stops elongation of the chain.
3. **Folic acid analogs.** The synthesis of both purines and pyrimidines requires folic acid as a carrier of C1 fragments. The transfer of a C1 fragment from tetrahydrofolic acid to an acceptor results in the oxidation of tetrahydrofolic acid to dihydrofolic acid. In order to recycle the coenzyme, the cell requires NADPH and dihdyrofolic acid reductase. There are a number of folic acid analogs, such as methotrexate and aminopterin, that specifically act as powerful competitive inhibitors of dihydrofolic acid reductase.

NOTE

A nucleotide analog lacking a 3′-C OH will terminate elongation when it is inserted; e.g., cytosine arabinoside or other "dideoxynucleotides."

DNA REPAIR

The structure of DNA can be altered in a number of ways. Incorrect bases may be inserted during replication or changes can result from exposure to ultraviolet radiation or chemicals found in the environment. Several repair systems have evolved that correct many of these changes.

A. **General terminology and definitions**

1. **Mutation** is a heritable change in the nucleotide sequence of DNA.
2. **Mutagen** is a chemical that alters the structure of a base in DNA.
3. **Point mutation** is a change in a single nucleotide. There are two types of point mutations, transitions and transversions.
   a. **Transition** is the change of one purine for another purine or of one pyrimidine for another pyrimidine. Transitions occur either from mispairing of bases during DNA replication or from chemical insult. For example, nitrous oxide converts cytosine to uracil by the removal of the amino group on the cytosine ring.
   b. **Transversion** is the exchange of a purine for a pyrimidine or vice-versa.

B. **Types of DNA repair.** Many of the mistakes occurring during replication are corrected by the proofreading (3′ to 5′ exonuclease) activity of DNA polymerases. Additional types of repair mechanisms exist, which correct alterations that occur due to chemical or environmental insult.

1. **Nucleotide excision-repair mechanism of UV-damaged DNA.** Ultraviolet light induces the formation of dimers between adjacent pyrimidines in DNA (usually between adjacent thymines). The formation of pyrimidine dimers prevents DNA replication and normal gene expression. As shown in Figure 3-3, four steps are involved in repairing this damage.
   a. **Incision.** A UV-specific endonuclease recognizes the damaged DNA and makes a nick in the phosphodiester backbone several bases away on each side of the dimer.
   b. **Removal.** A helicase in the same enzyme complex removes the oligonucleotide.
   c. **Polymerization.** DNA polymerase fills in the gap by adding nucleotides, starting with the addition of the first nucleotide to the free 3′ OH group created by the incision and moving in a 5′ to 3′ direction.

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Nucleotide excision repair removes a UV dimer and replaces it with normal nucleotides.

d. **Ligation.** DNA ligase forms a phosphodiester bond between the new DNA segment and the original DNA strand.

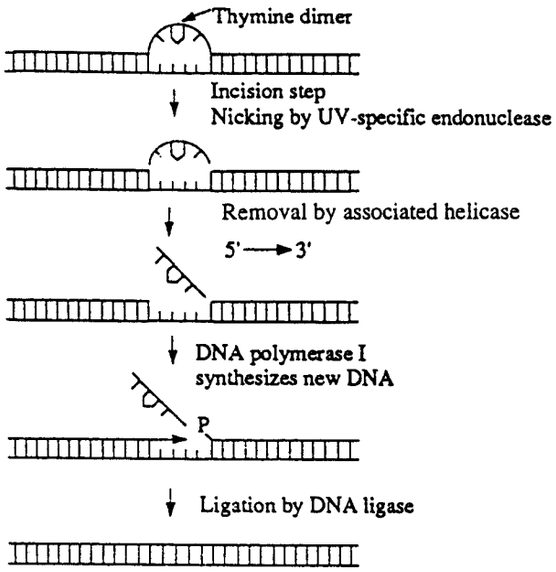

Figure 3-3. Nucleotide excision-repair mechanism for UV-damaged DNA.

2. **Base excision repair.** The most common base transition is the deamination of cytosine to uracil in the DNA duplex. The deamination leaves an improper base pair between the two strands (U-G instead of C-G). The repair mechanism for this type of damage involves the following steps.
   a. **Removal of the uracil base.** A uracil-DNA glycosidase recognizes the mismatch and hydrolyzes the N-glycosidic bond between uracil and the deoxyribose moiety, leaving an apyrimidinic site (AP site) in the DNA strand.
   b. **Cleavage of phosphodiester.** A specific endonuclease recognizes the damage and nicks the phosphodiester bonds adjacent to the missing base, thus releasing dexoyribose and creating a gap. Endonucleases that cleave at apyrimidinic sites (or apurinic sites) belong to a family known as AP nucleases.
   c. **Insertion of new cytosine nucleotide and ligation.** DNA polymerase fills the gap, and the nick is sealed by DNA ligase.

C. **Diseases associated with DNA repair.** Since repair mechanisms play such an important role in mutation surveillance and prevention, inherited defects that alter the activities of the repair enzymes can lead to dramatic increases in frequency of muta-

tions. Several autosomal recessive diseases such as xeroderma pigmentosa, ataxia telangiectasia, Bloom syndrome, and Fanconi anemia are associated with either documented or suspected defects in DNA repair mechanisms. All of these diseases occur with high frequency and are associated with a predisposition to malignancy.

1. **Xeroderma pigmentosa** is an autosomal recessive trait, characterized by extreme sensitivity to sunlight, skin changes and a predisposition to malignancy. The disease results from a defective excision-repair mechanism for UV-damaged DNA.
2. **Ataxia telangiectasia** is an autosomal recessive disorder, characterized by hypersensitivity to ionizing radiation, degenerative ataxia, dilated blood vessels, chromosome aberrations, and lymphomas. The disease results from a defect in AP endonuclease.

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- *An autosomal recessive deficiency in nucleotide excison repair results in xeroderma pigmentosa.*
- *An autosomal recessive deficiency in base excision repair results in ataxia telangiectasia.*

Transcription

The process of making an RNA copy of DNA is called transcription, and is catalyzed by the enzyme RNA polymerase. In any particular region of the DNA double helix, only one of the two DNA strands is transcribed into RNA. The RNA strand is always synthesized in a 5′ to 3′ direction, but from an antiparallel and complementary DNA template strand. The completed RNA is therefore antiparallel and complementary to the template or "anti-sense" DNA strand. The RNA is, however, parallel and identical to the "sense" strand of the DNA duplex (except that it uses uracil instead of thymine). Cells contain three types of RNA, each having a different function. Messenger RNA (mRNA) carries the information specifying the amino acid sequence of proteins. Transfer RNA (tRNA) and ribosomal RNA (rRNA) play important roles in protein synthesis. This chapter will review the key structural features of the genes coding for the three different types of RNA, the transcriptional machinery, and the steps involved in the process of transcription.

STRUCTURE AND TRANSCRIPTION OF PROKARYOTIC GENES

In prokaryotes, both the gene structure and the machinery for transcription are considerably simpler than in eukaryotic systems.

A. **DNA elements.** Specific sequences in the DNA act as start and stop signals for transcription.

1. **Bacterial promoter region.** The region of DNA that binds RNA polymerase and allows transcription to be initiated is known as the promoter region. As shown in Figure 4-1, the promoter region contains a starting point at which transcription is initi-

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Most E. coli promoters contain two recognition sequences for RNA polymerase:

- *Pribnow sequence at -10*
- *-35 sequence*

ated and two consensus sequences that are recognized by RNA polymerase. The sequences at which RNA polymerase bind are located at positions -10 and -35. By convention, the starting point is designated as +1. (Negative numbers are not transcribed and indicate that the nucleotide is upstream from the initiation site, i.e., on the 5' side of the starting site).

a. **Pribnow box.** The Pribnow box is an A-T rich sequence (TATATT) located 10 base pairs upstream from the site at which transcription begins. This sequence is present in almost all promoters and is involved in the initial unwinding of DNA by RNA polymerase.
b. **Hexamer at -35 position.** Another consensus sequence of six nucleotides is located 35 base pairs upstream from the start site for transcription. This TTGACA sequence is involved in the initial recognition of the promoter by RNA polymerase.
c. **Start site.** The first nucleotide transcribed into RNA is usually a purine (Pu), either A or G. The next two nucleotides (designated NN) can be any of the four nucleotides found in RNA (A, G, C, or U).

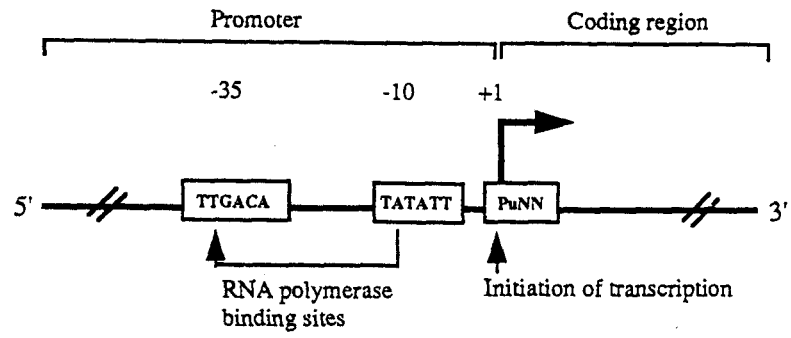

Figure 4-1. Bacterial promoter.

2. **Bacterial terminators.** Termination of transcription involves the release of both the DNA template and the newly synthesized RNA from RNA polymerase. Bacterial RNA polymerase has two methods of termination.
   a. **Rho-independent termination** occurs when the newly synthesized RNA folds back on itself and forms a hairpin loop that is stabilized by hydrogen bonding between complementary bases. The hairpin loop must be followed by a run of 6-8 U residues that form weak bonds with the complementary run of 6-8 A residues in the DNA template (Figure 4-2). These two structural features of the newly synthesized RNA promote dissociation of the RNA from the DNA template.

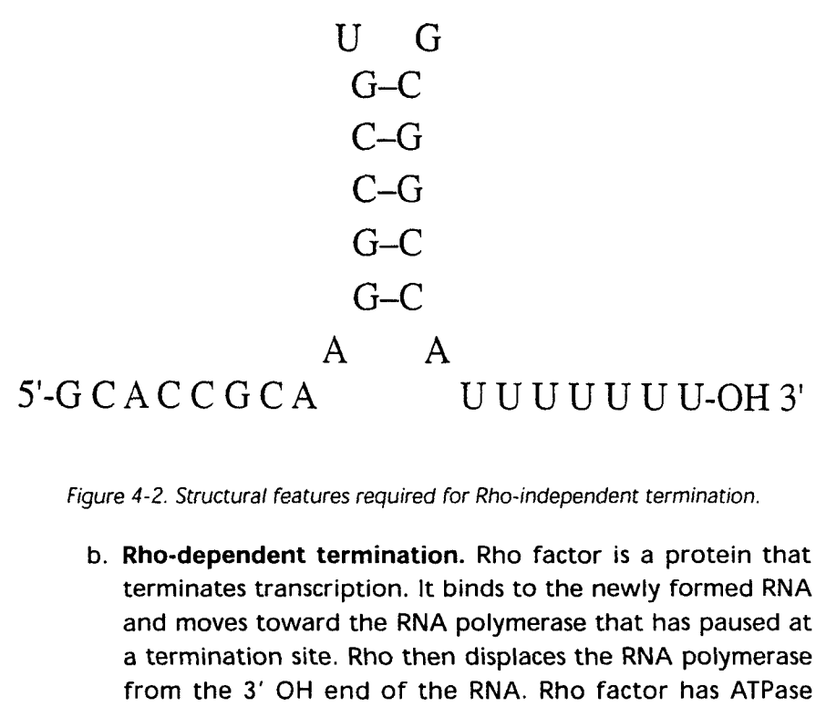

Figure 4-2. Structural features required for Rho-independent termination.

b. **Rho-dependent termination.** Rho factor is a protein that terminates transcription. It binds to the newly formed RNA and moves toward the RNA polymerase that has paused at a termination site. Rho then displaces the RNA polymerase from the 3′ OH end of the RNA. Rho factor has ATPase activity that is RNA dependent, and it requires a polyribonucleotide that is greater than 50 bases long.

B. **Transcriptional machinery.** In prokaryotic systems, all forms of RNA are synthesized by a single RNA polymerase.

1. **Structure of RNA polymerase.** This multisubunit enzyme exists in two forms, a core enzyme and a holoenzyme. The core enzyme has four subunits ($\alpha_2\beta\beta'$). It is capable of polymerizing ribonucleotides but it does not recognize promoter regions in the DNA. The holoenzyme has an additional sigma σ-subunit that allows the enzyme to recognize promoter sequences.
2. **Requirements for RNA synthesis.** The synthesis of RNA requires all four ribonucleotides (ATP, GTP, CTP, and UTP), a divalent cation (either Mg^{2+} or Mn^{2+}), and a template. Double-stranded DNA is the preferred template, but single-stranded DNA can also be used. Synthesis occurs in the direction of 5′ to 3′, and each time a nucleotide is added to the 3′ OH end, pyrophosphate is released. RNA polymerase does not require a primer and cannot proofread and correct mistakes.

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Rho-independent termination requires a specific 2′ helical stem to form in the newly-transcribed RNA; rho-dependent termination requires rho protein to move along the newly-transcribed RNA and to "catch" the RNA polymerase.

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RNA synthesis:

- *Requires an antiparallel and complementary template*
- *Occurs 5′ to 3′*
- *Is not proofread*
- *Can be initiated de novo (no primer is required)*

3. **Function of RNA polymerase subunits.** The α subunits are involved in binding to the consensus sequences in the DNA promoter region. The β subunit contains the active site that binds nucleotide triphosphates and forms the phosphodiester bond of the polyribonucleotide chain. The β′ subunit plays a role in the attachment of the enzyme to the DNA template. Sigma factor recognizes promoter sequences and provides the enzyme with specificity.

C. **Steps involved in transcription.** Transcription in prokaryotes involves three steps: initiation, elongation, and termination.

1. **Initiation.** The sigma subunit of the holoenzyme recognizes consensus sequences in the promoter, binds to DNA, and helps unwind the DNA double helix so that one strand can serve as a template. The initiating ribonucleotide triphosphate is usually a purine (either A or G). After the first few phosphodiester bonds are formed, the sigma subunit dissociates from the holoenzyme and the core enzyme begins elongation.
2. **Elongation.** The core enzyme moves along the template extending the RNA chain, and the region of local unwinding moves with it. As the enzyme leaves a region, the DNA duplex reforms and RNA is displaced as a growing polynucleotide chain. Growth of the chain is always in the 5′ to 3′ direction. RNA polymerase moves along the DNA template strand in the 3′ to 5′ direction.
3. **Termination.** The DNA template contains stop signals for transcription, as described above in paragraph A.2.

D. **Inhibitors of prokaryotic transcription.** Many antibiotics exert their action by interfering with bacterial RNA or protein synthesis while having no effect on eukaryotic RNA and protein synthesis.

1. **Rifampin** inhibits initiation of RNA synthesis.
2. **Actinomycin D** binds to DNA and inhibits RNA synthesis by blocking movement of RNA polymerase along the template.
3. **Streptolydigin** binds to the β subunit of RNA polymerase and blocks elongation.

NOTE

In prokaryotes, the processes of transcription and translation are coupled. Translation of mRNA starts prior to the completion of transcription. In these systems, almost all gene regulation occurs at the transcriptional level.

STRUCTURE AND TRANSCRIPTION OF EUKARYOTIC GENES

Both the structure of eukaryotic genes and the mechanisms for transcription are more complex than in prokaryotic systems.

A. **Transcriptional machinery: three RNA polymerases.** Each type of RNA is transcribed by a different RNA polymerase. The require-

ments of each of these enzymes are the same as for prokaryotic RNA polymerase. None of the RNA polymerases have the proofreading activities that DNA polymerases have.

1. **RNA polymerase I** is localized in the nucleolus and transcribes the genes for three types of ribosomal RNA: 28S, 18S, and 5.8S rRNA. rRNA is the most abundant RNA in the cell.
2. **RNA polymerase II** is found in the nucleoplasm, where it transcribes genes coding for proteins. The primary transcripts synthesized by RNA polymerase II are known as heterogeneous nuclear RNA (hnRNA), which are precursors for mRNA and snRNA (small nuclear RNAs). The snRNAs (U1-U5) are a part of the "spliceosome," a complex involved in removing intron sequences from precursor RNA molecules.
3. **RNA polymerase III** is located in the nucleoplasm and transcribes the genes for transfer RNA (tRNA) and 5S rRNA.

B. **Structure and transcription** of ribosomal RNA genes. The genes coding for rRNA are transcribed by RNA polymerase I in the nucleolus. The nucleolus contains large loops of DNA from several chromosomes, each containing a cluster of genes for rRNA. As shown in Figure 4-3, multiple copies of the genes for rRNA are tandemly arranged so that each gene is separated from the next by a spacer. The primary RNA transcript of each of these genes is a 45S RNA that serves as a precursor for 18S, 28S, and 5.8S rRNA. The primary transcript also contains spacer regions between each of the rRNAs. The spacer regions are removed by a series of endonucleolytic cleavages. Processing of the precursor rRNA results in equimolar amounts of each of three types of rRNA. Following the processing shown in Figure 4-3, the rRNA species are modified by methylation of specific 2'-OH groups. This reaction is believed to confer stability on the rRNA molecules and protect them from endonucleolytic cleavage after incorporation into ribosomes.

C. **Genes coding for protein.** RNA polymerase II is found in the nucleoplasm, where it transcribes the genes whose RNAs will be translated into proteins. RNA polymerase II cannot initiate transcription itself and is absolutely dependent on additional proteins known as transcriptional factors that act at promoter sites. The essential features of genes that are transcribed by RNA polymerase II are shown in Figure 4-4 and are briefly discussed.

1. **Start site.** The initial nucleotide in position +1 of the RNA transcript is usually A, flanked by pyrimidines.
2. **Promoters.** There are two common sequences in promoters that are utilized by RNA polymerase II.

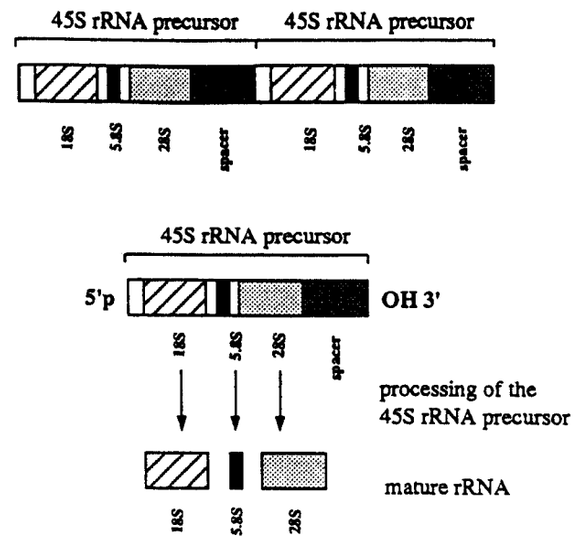

Figure 4-3. Structure and transcription of ribosomal RNA genes.

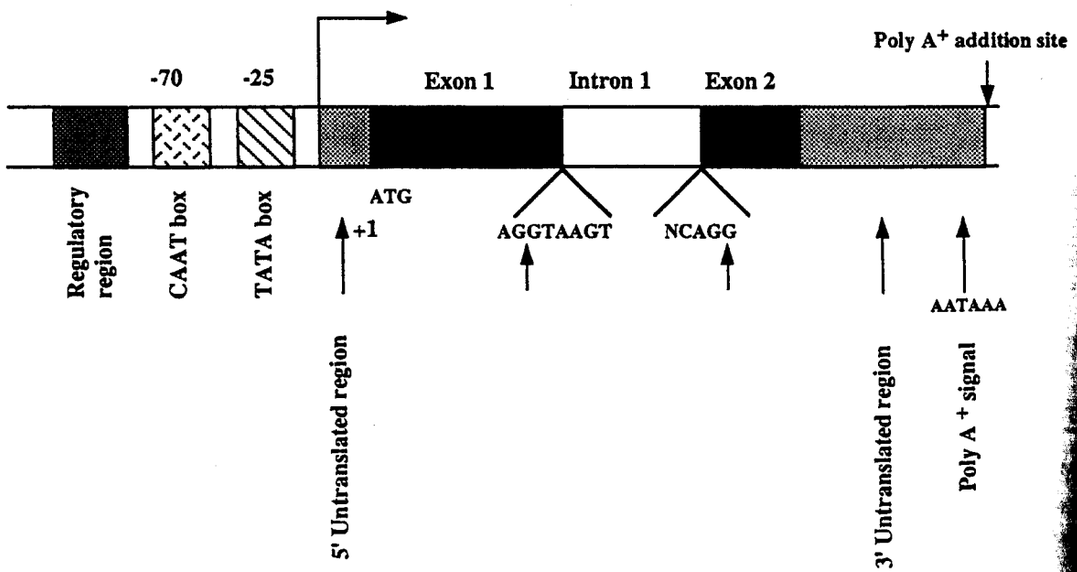

Figure 4-4. Structure of a typical gene transcribed by RNA polymerase II.

a. **TATA box** (Hogness box). This sequence of base pairs, which is important in the initiation of transcription, is found in all eukaryotes. It is located approximately 25 base pairs upstream (-25) from the start point, and is almost identical

to the Pribnow box found in bacterial systems. TFIID, the critical transcription factor for RNA polymerase II, binds here.

b. **CAAT box.** This sequence is usually found between position -75 and -80, but it can function at distances that vary considerably from the start point. Binding of transcription factors at this site may influence the formation of initiation complexes at other sites.

3. **Regulatory regions.** Sequences that either increase or decrease the rate at which transcription is initiated by RNA polymerase II exist at various places in the gene. Although they are usually located upstream from the start site, they may also be internal to the gene or downstream from the gene. Enhancer sequences bind transcription factors that increase the rate of transcription while silencer sequences bind factors that decrease the rate of transcription.

4. **Exon and introns.** The segment(s) of the gene that are maintained in the mature mRNA and code for protein are known as exons. Introns are the portions of the primary RNA transcript that are removed by splicing together the exons (Figure 4-5). The splicing usually occurs at a consensus sequence (GU.....AG) found at the boundaries between introns and exons (discussed in more detail below).

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RNA polymerase II initiation requires:

- *Specific transcription factors bound to enhancer sequences*
- *General transcription factors bound to promotor sequences*
- *Association of RNA polymerase II with transcription factors to form initiation complex*

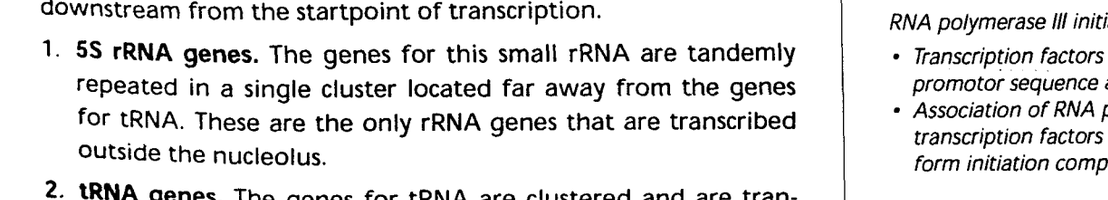

Figure 4-5. hnRNA and mature mRNA.

D. **Genes** coding for tRNA and 5S rRNA. These genes are transcribed in the nucleoplasm by RNA polymerase III. The promoters for both the 5S rRNA and the tRNA genes are internal and are found downstream from the startpoint of transcription.

1. **5S rRNA genes.** The genes for this small rRNA are tandemly repeated in a single cluster located far away from the genes for tRNA. These are the only rRNA genes that are transcribed outside the nucleolus.

2. **tRNA genes.** The genes for tRNA are clustered and are transcribed as larger precursor RNA molecules, which are processed by endonucleases. Internal promoters are found in two regions downstream from the start site. tRNA is subject to a number of post-transcriptional modifications, including modification of specific bases and the addition of the sequence -CCA to the 3' OH of the tRNA.

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RNA polymerase III initiation requires:

- *Transcription factors bound at internal promotor sequence and at start site*
- *Association of RNA polymerase III with transcription factors at start site to form initiation complex*

PROCESSING OF EUKARYOTIC RNA

The heterogeneous nuclear RNAs (hnRNAs), synthesized by RNA polymerase II, leave the nucleus as messenger RNAs (mRNAs). The processing of the hnRNA starts while transcription is still occurring and involves covalent modification of both the 5' and 3' ends, followed by cutting and splicing to eliminate the intervening sequences that separate the coding regions.

A. **5' Capping.** The 5' end of the RNA is "capped" shortly after the initiation of RNA synthesis. This process involves the addition of an "inverted" methylated guanosine molecule to the first nucleotide in the RNA transcript. The 7-methyl-guanosine is linked through a 5'-5' triphosphate linkage. The 5' cap plays an important role in the initiation of protein synthesis and protecting the mRNA chain from degradation.

B. **Polyadenylation of the 3' OH end.** Mature mRNA molecules have a poly-A tail that is between 20 and 250 nucleotides long. The tail is added to hnRNA by the enzyme poly-A polymerase. A consensus sequence near the end of the gene provides a signal that initiates polyadenylation. Polyadenylation is believed to increase the stability of hmRNA. Not all mRNA molecules are polyadenylated: histone mRNAs, for example, have no poly-A tails.

C. **RNA splicing.** hnRNA contains coding sequences (exons) that are separated from one another by intervening sequences (introns). In the conversion of hnRNA to mature mRNA, the introns are removed and the exons are spliced together. This process is illustrated in Figure 4-6. During the excision of introns, the 5' cap and the poly-A tail are not removed. The base sequence at the beginning of an intron is GU... and at the end of an intron is ...AG. This consensus sequence defines the sites at which cutting and splicing occur. The intron is excised as a loop (lariat) of RNA that is degraded. Following excision, ligation of the exons occurs. These reactions occur in the nucleus.

NOTE

Since, in eukaryotes, transcription and translation are not coupled, the primary transcript (hnRNA) is extensively modified in the nucleus, yielding mature mRNA that is then transported to the cytoplasm for translation into protein.

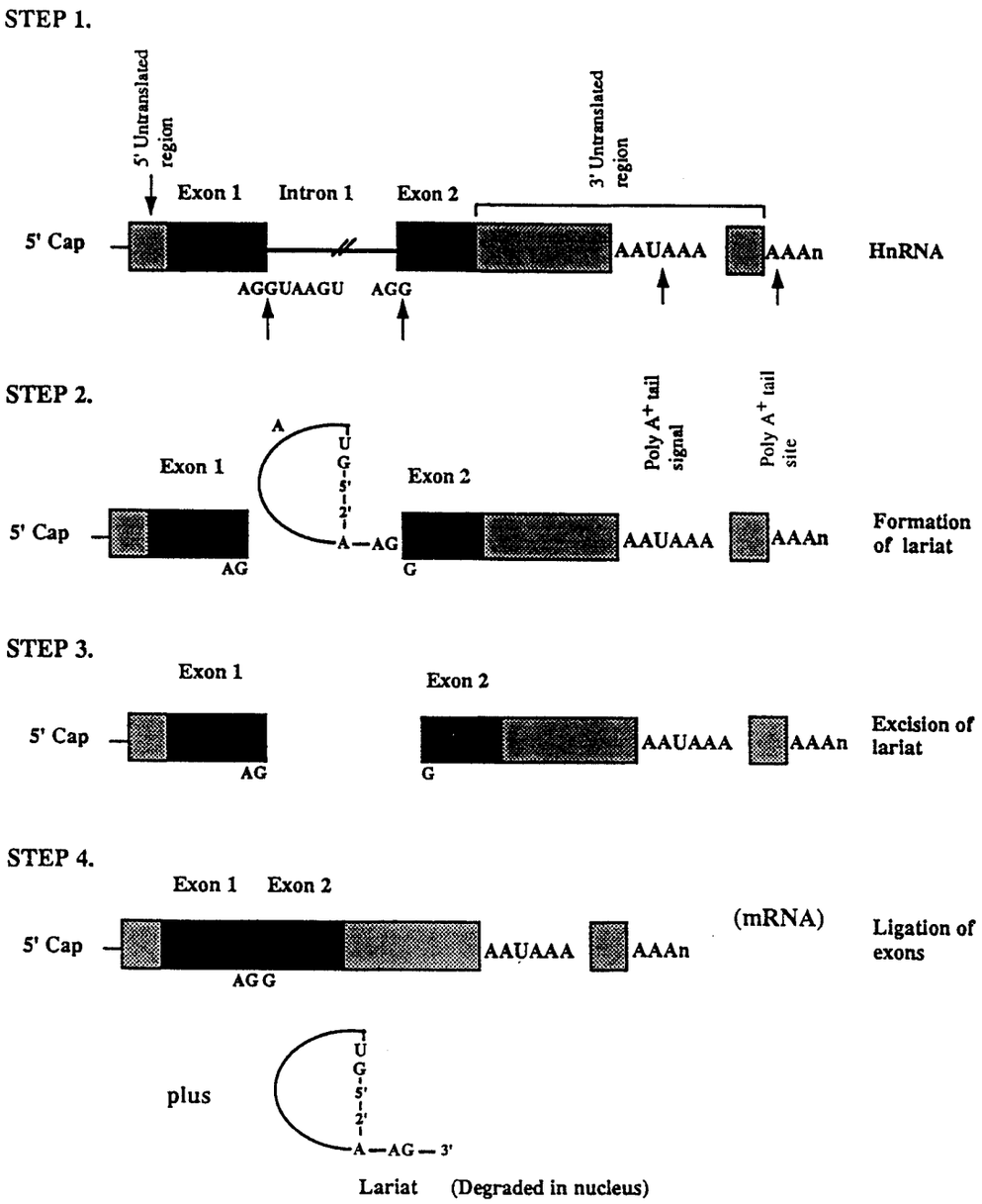

Figure 4-6. Processing of hnRNA.

Protein Synthesis

Translation is the process by which the base sequence in mRNA is decoded into an amino acid sequence. All three types of RNA play different and essential roles in translation. The genetic code is defined as the relationship between the sequence of bases in DNA (or its RNA transcript) and the sequence of amino acids in proteins. mRNA is the template for protein synthesis and acts as a "working copy" of the gene in which the code words for each amino (codons) have been transcribed from DNA to mRNA. The tRNA has a three-base anticodon that hydrogen bonds with the complementary codon in mRNA, thus aligning amino acids in the appropriate sequence prior to peptide bond formation. Ribosomes are complexes of protein and rRNA that serve as the molecular machines, coordinating the interactions between mRNA, tRNA, and the enzymes and proteins factors required for protein synthesis. This chapter will review the structure and function of the three types of RNA, the genetic code, the structure and function of ribosomes, and the events involved in protein synthesis. Commonly used antibiotics that inhibit protein synthesis will also be reviewed.

ROLE OF RNA IN PROTEIN SYNTHESIS

Each of the three major types of RNA plays an essential role in protein synthesis.

A. **Messenger RNA (mRNA)** acts as the "working copy" of the gene coding for a protein. The mRNA carries the information from the genome in the nucleus to the cytosol where protein synthesis occurs. Messenger RNA is synthesized as an hnRNA precursor and is processed to mature mRNA in the nucleus. The

mature mRNAs are capped at their 5' end with 7-methylguanosine attached through a triphosphate linkage to the first nucleotide in the mRNA. Most mRNAs also contain a poly-A tail attached to the 3' end. Messenger RNA constitutes approximately 5% of the total cellular RNA and has a shorter half-life than other types of RNA. The length of the mRNA is related to the size of the gene. The key structural features of mRNA are shown in Figure 5-1.

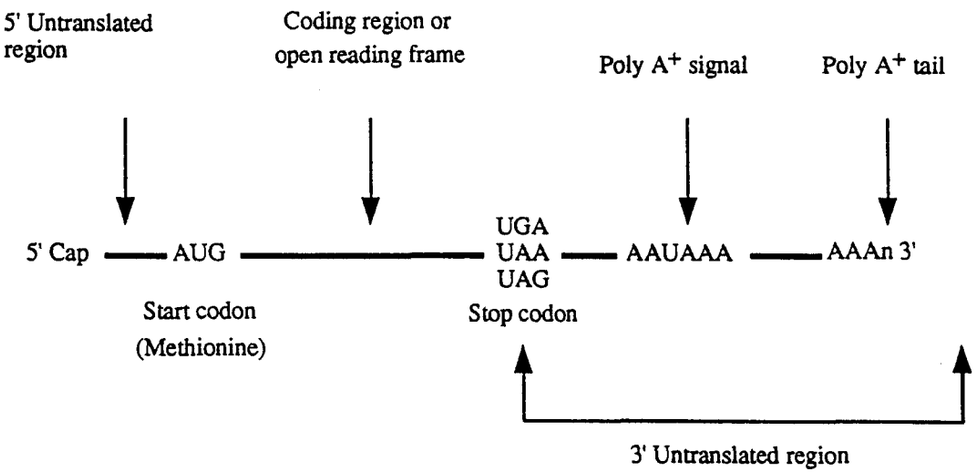

Figure 5-1. Structure of eukaryotic mRNA.

B. **Ribosomal RNA (rRNA).** Ribosomal RNA is the most abundant form of RNA, comprising approximately 80% of the cellular RNA. Ribosomes, the machines for synthesizing protein, are complexes containing protein and rRNA. In prokaryotic systems there are three forms of rRNA: 23S, 16S, and 5S rRNA, which vary in length from 120 to 3700 nucleotides. Eukaryotic rRNA has four forms of rRNA: 28S, 18S, 5.8S, and 5S. In eukaryotic systems, all of the forms of rRNA except 5S rRNA are synthesized in the nucleolus. The precise function of rRNA is unclear, but it is necessary for the organization of a functional ribosome.

C. **Transfer RNAs (tRNAs)** are the adaptor molecules in protein synthesis. They have a three-base region (anticodon region) that recognizes and hydrogen bonds to the complementary codon in mRNA, which specifies a particular amino acid. The tRNAs react with amino acids at their 3' ends. Transfer RNAs are small, containing approximately 80 nucleotides. The key structural features of tRNA are shown in Figure 5-2 and are described briefly below.

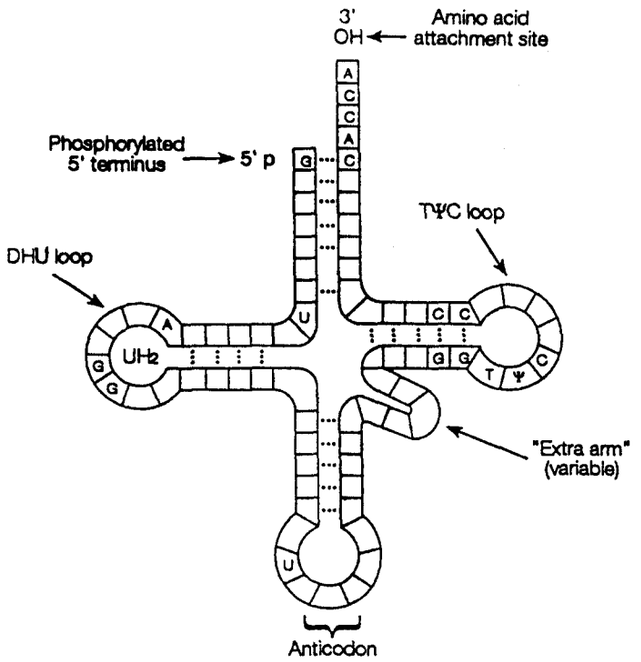

Figure 5-2. Structure of tRNA.

1. The 5' end of tRNA is a monophosphate, rather than a triphosphate, and the base is usually G.
2. The 3' end of tRNA has the sequence CCA. The "activated amino acid" is covalently attached to the 3' OH of the terminal adenosine. The bond linking the amino acid to tRNA is a high-energy bond that provides energy for peptide bond formation, an endergonic reaction.
3. Unique structural features of tRNA include three loops and an "extra arm" created by internal hydrogen bond formation. The anticodon loop contains three bases that are antiparallel and complementary to the bases in the codon of mRNA.
4. A high degree of secondary structure is found in tRNA due to the bending back of the molecule on itself with the formation of internal base pairs that stabilize the secondary structure. About half of the nucleotides in tRNA are base paired to form double helices.
5. A large number of unusual bases are found in tRNA that are not found in other types of RNA. These are believed to be important in maintaining the characteristic secondary structure. Inosine, pseudouridine, dihydrouridine, ribothymidine and methylated guanosine are found exclusively in tRNA.

NOTE

The 5' end of an RNA that has been cut out of a larger precursor RNA will be a monophosphate; the 5' end of a precursor or unprocessed RNA will be a triphosphate.

NOTE

The CCA amino acid attachment site of tRNA is encoded in prokaryotes, but is added post-transcriptionally in eukaryotes.

THE GENETIC CODE

The genetic code is the relationship between the sequence of bases in DNA (or its working RNA transcript) and the sequence of amino acids in proteins.The genetic code is read in triplets. The code words are almost (but not absolutely) universal. Human mitochondria have a few differences in codons.

A. **Codon** is a group of three nitrogenous bases (base triplet) that represents an amino acid or signals the initiation or termination of protein synthesis. The codons in mRNA are antiparallel and complementary to the anticodons (recognition sites) found in tRNA. The codons in mRNA are shown in Table 5-1. There are 64 possible combinations of the four bases in mRNA, and thus the genetic code contains 64 codons.

B. **Amino acid codons.** There are 61 triplets that specify 20 amino acids. (The remaining three codons are "stop" codons.) Since more than one codon can specify the same amino acid, the genetic code is said to be redundant or degenerate. Different codons for the same amino acid usually differ in the third base.

C. **Stop codons.** There are only three codons that do not specify an amino acid. These codons (UAA, UAG, UGA) act as termination signals during protein synthesis.

D. **Start codon**. AUG (which codes for methionine) and sometimes GUG are signals for the initiation of translation. In prokaryotic systems, polypeptide chains start with a modified amino acid, formylmethionine (fMet). Prokaryotes have a specific tRNA that carries fMet and recognizes the initiating AUG codon in mRNA. In eukaryotic systems, the AUG closest to the 5′ end of the mRNA is the start signal for protein synthesis. Eukaryotes also have a specific initiating tRNA that carries Met.

first base	second base: U	second base: C	second base: A	second base: G	third base
U	UUU, UUC: Phe; UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr; UAA: Stop; UAG: Stop	UGU, UGC: Cys; UGA: Stop; UGG: Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His; CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile; AUG: Start/Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn; AAA, AAG: Lys	AGU, AGC: Ser; AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp; GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

Table 5-1. The genetic code.

PROKARYOTIC AND EUKARYOTIC RIBOSOME STRUCTURE

The structure and function of ribosomes are very similar in prokaryotes and eukaryotes.

A. **Common features.** *E. coli* and eukaryotic ribosomes share the following features.

1. **Function.** Ribosomes are the site at which protein synthesis occurs.
2. **Components.** Protein and rRNA are the building blocks for ribosomes. Each of the forms of rRNA and most of the proteins are present in only one copy per ribosome.
3. **Subunits.** As shown in Figure 5-3, ribosomes have two subunits, one large and one small. (Note that the Svedberg units (S) are not additive).

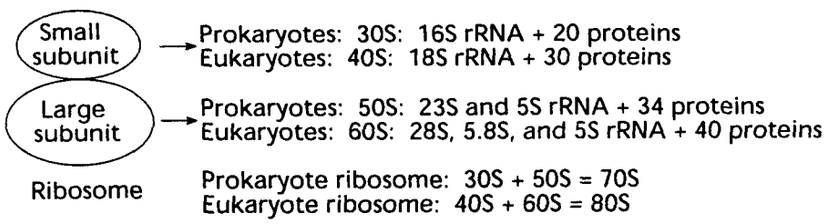

Figure 5-3. Composition of ribosomes.

B. **Differences in prokaryotic and eukaryotic ribosomes.** As seen in Figure 5-3, eukaryotic ribosomes are larger than prokaryotic ribosomes. In prokaryotes, the 70S ribosome is made up of a large subunit (50S) and a small subunit (30S), whereas eukaryotes have an 80S ribosome containing a large subunit (60S) and a small subunit (40S). Eukaryotic ribosomes contain four forms of rRNA, while prokaryotes have only three forms. Other minor differences are found in the number of proteins found in both the large and small subunits of eukaryotic and prokaryotic systems.

C. **Eukaryotic ribosome assembly.** In eukaryotes, the large ribosomal subunit (60S) is assembled in the nucleolus, while the small subunit (40S) is assembled in the nucleus. The ribosomal proteins are synthesized in the cytoplasm and are transported into the nucleus, where they combine with the appropriate rRNA species to form the large and small ribosomal subunits. After assembly, the ribosome moves through the nuclear pores into the cytoplasm by an unknown mechanism. Since prokaryotes have no defined nucleus, all of these processes occur in the cytoplasm.

NOTE

In general, the small subunit is necessary for initiating protein synthesis; the large subunit catalyzes the elongation steps.

ACTIVATION OF AMINO ACIDS AND ATTACHMENT TO tRNA

IN A NUTSHELL

Each tRNA muct be "charged" with the correct amino acid by a separate tRNA synthetase.

The formation of peptide bonds between amino acids is an endergonic process and therefore requires a source of energy. The energy is derived from ATP and is transferred to the bond that links the amino acid to the 3' OH group of the tRNA molecule. Enzymes that "activate" the carboxyl group of amino acids are known as aminoacyl-tRNA synthetases. These enzymes are highly specific for both the amino acid and the tRNA. There is a specific enzyme for each amino acid. As shown in Figure 5-4, the attachment of the amino acid to the tRNA occurs in a two-step process.

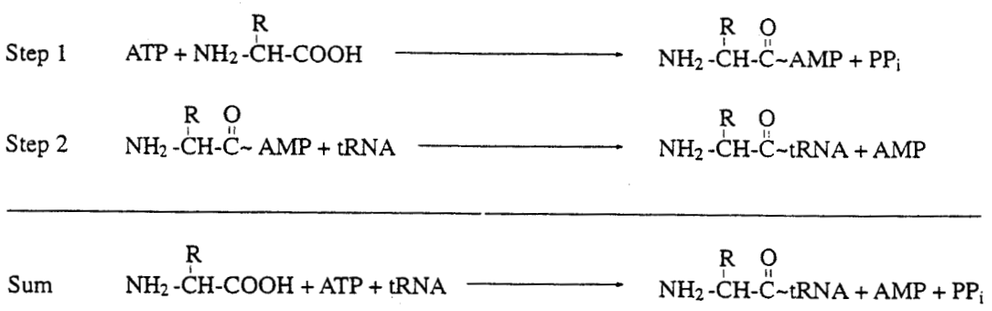

Figure 5-4. Formation of aminoacyl-tRNA.

The ΔG° for this reaction is close to zero, but in the cell, the overall reaction is highly exergonic and essentially irreversible because the high-energy bond in the pyrophosphate product is rapidly hydrolyzed by pyrophosphatases.

STEPS INVOLVED IN TRANSLATION

The process of translating the genetic code found in mRNA into a specific sequence of amino acids in a protein involves three steps: initiation, elongation, and termination. Protein synthesis occurs in the direction of amino terminal to carboxy terminal, and the mRNA is read from the 5' end to the 3' end.

A. **Initiation of protein synthesis.** Initiation of protein synthesis requires special tRNA molecules and the formation of an initiation complex.

   1. **Initiating tRNA molecules.** The codon AUG in mRNA usually signals the beginning of protein synthesis. AUG is the codon for methionine.

a. **Prokaryotes.** The initial methionine residue has a formyl group attached to it (fMet). The initiating tRNA is known as $tRNA^{fMet}$. The tRNA that carries methionine to any other position is known as $tRNA^{Met}$.

b. **Eukaryotes.** The initiating methionine has no formyl group. The initiating tRNA is known as $tRNA_i^{Met}$, while the tRNA involved in elongation of the protein is known simply as $tRNA^{Met}$.

2. **Formation of initiation complex.** The assembly of the 70S initiation complex found in prokaryotes is a stepwise process involving the formation of an intermediate 30S complex.

a. **Assembly of the 30S complex.** Formation of the 30S complex requires mRNA, the 30S ribosomal subunit, three initiation factors (IF-1, IF-2, and IF-3), GTP, and $tRNA^{fMet}$. The mRNA contains the start codon (AUG or GUG) that recognizes $tRNA^{fMet}$. There is a special sequence of bases upstream from the AUG codon called the Shine-Dalgarno sequence. This sequence of bases binds with the 16S rRNA in the 30S ribosomal subunit. The binding of the mRNA to the 30S ribosomal subunit requires the transient help of IF-3. After mRNA binds to the 30S ribosomal subunit, IF-3 dissociates. The complex between mRNA and the 30S ribosomal subunit then binds IF-2, GTP, and $tRNA^{fMet}$ to produce an active 30S initiation complex. This reaction requires IF-1.

b. **Assembly of the 70S initiation complex** occurs by the interaction of the 50S ribosomal subunit with the 30S complex. The formation of the 70S complex results in the hydrolysis of GTP to GDP and P_i, as well as the dissociation of both IF-1 and IF-2. The structure of the 70S initiation complex is shown in Figure 5-5. The association of the ribosomal subunits creates two important sites for protein synthesis within the ribosome, the A site and the P site.

(1) The **aminoacyl site (A site)** binds the incoming tRNA molecule carrying an activated amino acid.

(2) The **peptidyl site (P site)** is the site on the ribosome at which $tRNA^{fMet}$ initially binds. After formation of the first peptide bond, the P site is occupied by the growing peptide chain. The $tRNA^{fMet}$ recognizes two sites, the P site on the ribosome and the start site (AUG) on the mRNA.

B. **Elongation of the protein.** This process is a three-step cycle that is illustrated in Figure 5-5. Each step is described below.

1. **Binding of aminoacyl-tRNA to the A site.** The A site will always contain the next amino acid to be added to the peptide chain.

IN A NUTSHELL

In prokaryotes, the initiating AUG is defined by its position near a Shine-Dalgarno sequence. Therefore, prokaryotic mRNAs may contain multiple genes ("polycistrionic"). Since each gene in the mRNA begins with a Shine-Dalgarno + AUG sequence, each will be independently translated by ribosomes.

In eukaryotes, the ribosomal small subunit (with $tRNA^{Met}$ + eIF-2 + GTP bound) must begin each time at the 5' cap, then scan the mRNA for the first AUG to initiate, where it is joined by the large subunit. Eukaryotic mRNAs are typically monocistronic (one gene per mRNA).

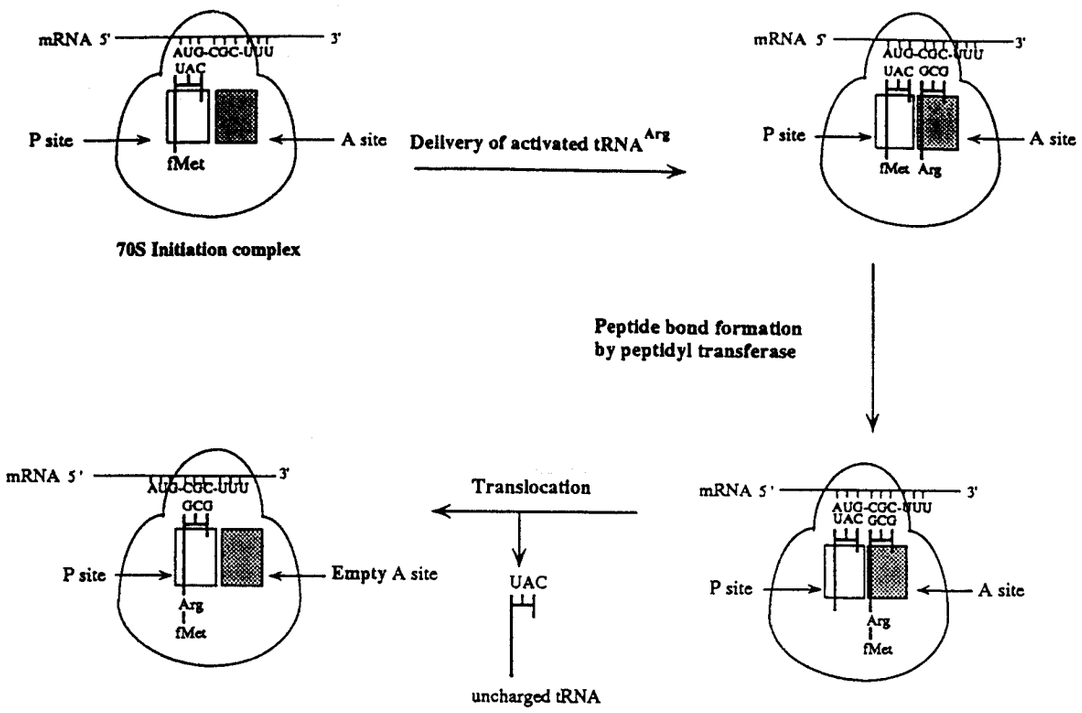

Figure 5-5. Elongation phase of protein synthesis in bacteria.

The specific aminoacyl-tRNA that comes into the A site is determined by the mRNA codon that is positioned above the A site. Thus, in Figure 5-5, $tRNA^{Arg}$ is delivered to the A site because CGC codes for Arg (Table 5-1). The delivery of the aminoacyl-tRNA to the A site requires elongation factor EF-Tu and the hydrolysis of GTP to GDP and P_i. GDP remains associated with EF-Tu until it is displaced by another elongation factor, EF-Ts. The new Tu-Ts complex is dissociated by the binding of a second GTP to give GTP-Tu complex.

2. **Peptide bond formation.** Formation of a peptide bond between the amino acid (or peptide) in the P site and the amino acid in the A site is catalyzed by peptidyl transferase, which is an integral part of the 50S ribosomal subunit. This reaction results in the release of the amino acid from the tRNA in the P site. The resulting peptide is bound to the tRNA in the A site.
3. **Translocation.** In order for elongation to continue, translocation has to occur. This involves three movements. First, the uncharged tRNA leaves the P site. Next, the peptidyl tRNA moves from the A site to the P site. And finally the mRNA moves a distance of three nucleotides to bring a new codon

NOTE

During peptide bond formation, the amino group of the amino acid bound to the A site tRNA is bound to the α-carboxy group of the amino acid bound to the P site tRNA.

into a position above the empty A site. The movement of mRNA requires an elongation factor known as translocase (EF-G). Hydrolysis of GTP is required to release EF-G from the ribosome. At some point during elongation of the peptide chain, the formyl group is removed from the initial methionine residue.

C. **Termination of protein synthesis.** When any one of the three stop codons is encountered, elongation is terminated. Cells do not have tRNAs with anticodons that are complementary to stop codons. Release of the peptide from the ribosome requires a protein known as a release factor (RF) that binds to the stop codon. Hydrolysis of the peptidyl-tRNA bond requires an enzyme, peptidyl-tRNA hydrolase, and GTP, which is hydrolyzed to GDP and P_i. The polypeptide leaves the ribosome, the ribosome dissociates into its 30S and 50S subunits, and the mRNA is released.

IN A NUTSHELL

Elongation is catalyzed by the large subunit:

- *Charged tRNA binds to the A site (requires GTP hydrolysis)*
- *Peptide bond formation*
- *Translocation (requires GTP hydrolysis)*

INHIBITORS OF PROTEIN SYNTHESIS

Several antibiotics recognize differences between prokaryotic and eukaryotic protein synthesis and can be used to inhibit one independent of the other. Additionally, some bacterial toxins inhibit protein synthesis in animal cells. Table 5-2 lists several inhibitors of protein synthesis.

Inhibitor	Prokaryote or eukaryote	Step/site of action
Tetracycline	Prokaryotes	Initiation; prevents binding of aminoacyl-tRNA to ribosome
Streptomycin	Prokaryotes	Initiation; causes misreading of code
Erythromycin	Prokaryotes	Translocation
Chloramphenicol	Prokaryotes	Ribosomal peptidyl transferase
Puromycin	Both	Elongation; binds to A site and prematurely terminates chain growth
Cyclohexamide	Eukaryotes	Ribosomal peptidyl transferase
Sparsomycin	Eukaryotes	Initiation; inhibits formation of initiation complex
Ricin	Eukaryotes	Initiation; binds to 60S subunit
Diphtheria toxin	Eukaryotes	Translocation; inactivation of EF-2
Pseudomonas toxin	Eukaryotes	Translocation; inactivation of EF-2

Table 5-2. Inhibitors of protein synthesis.

Regulation of Gene Expression

Regulation of gene expression is an essential feature in maintaining the functional integrity of a cell. Regulation of a gene may occur in a variety of ways, some of which are positive while others are negative. Regulation of gene expression in prokaryotes almost always involves either initiation or termination of transcription. In eukaryotes, transcription is more complicated than in prokaryotes and, therefore, there are more possible sites for regulation. In both prokaryotes and eukaryotes, transcriptional regulation is usually achieved by the interaction of proteins with specific sequences in the DNA, resulting in either an increase or a decrease in the rate of transcription.

REGULATION OF TRANSCRIPTION IN PROKARYOTES

The regulation of gene expression in prokaryotes is usually achieved by regulating the rate at which transcription is initiated. The examples that will be considered are the positive and negative control of the lac operon in *E. coli.*

A. **Operon concept.** In bacteria, a set of structural genes and a regulatory region constitute an operon. The structural genes code for a group of proteins required for a particular metabolic function. The regulatory region is upstream (to the 5' side) of the structural genes. The structural genes in an operon are coordinately regulated, i.e., the expression of all the structural genes is controlled by the same regulatory region in the DNA.

B. **Description of the lac operon.** The arrangement of the structural and regulatory genes in the lactose (lac) operon of *E. coli* is shown in Figure 6-1. The regulatory gene (i) codes for a repressor that can interact with the operator sequence (O). The operator

sequence is situated adjacent to the three structural genes (Z, Y, and A) that code for three enzymes concerned with lactose metabolism (β-galactosidase, permease, and transacetylase). The expression of these three genes is regulated by the operator sequence. RNA polymerase binds at the promoter site (P).

1. **Negative regulation of the lac operon.** In the absence of lactose, the repressor protein binds to the operator sequence and blocks the movement of RNA polymerase along the template, thus inhibiting transcription (Figure 6-1A). When lactose is present, it induces transcription by the following mechanism: Lactose is converted to 1,6-allolactose, a molecule that binds to the repressor and changes its conformation so that it no longer binds to the operator region. Thus, the repressor is released and the three structural genes are transcribed as a single mRNA that codes for all three proteins (Figure 6-1B). Messenger RNA coding for more than one protein is known as a polycistronic mRNA, and is found only in prokaryotic systems. In this model of negative regulation of an operon, a protein acts as the repressor and lactose (or 1,6-allolactose) acts as the inducer of transcription.
2. **Positive regulation of the lac operon.** The function of β-galactosidase in lactose metabolism is to cleave lactose to glucose and galactose. The galactose is ultimately converted to glucose by other enzymes. Therefore, if the bacteria has both glucose and lactose in the growth medium, there is no reason to activate the lac operon. When glucose is low, the lac operon is activated and the bacteria begins to use lactose to generate glucose. The effects of glucose are mediated by cAMP. When glucose is low, cAMP concentration is high and vice-versa. When cAMP increases, it binds to a protein known as CAP (catabolite activator protein). The CAP-cAMP complex activates transcription of the lac operon by binding to the promoter region and allowing RNA polymerase to initiate transcription. Thus, the CAP-cAMP complex is a positive regulator of the lac operon.

NOTE

Each structural gene in the polycistronic lac mRNA has a Shine-Dalgarno sequence to define its initiating AUG.

NOTE

The lac repressor is made constitutively, i.e., whether or not lactose is present.

IN A NUTSHELL

Full transcription of the lac operon requires:

- *Absence of the repressor (negative regulator)*
- *Presence of the activator (positive regulator)*

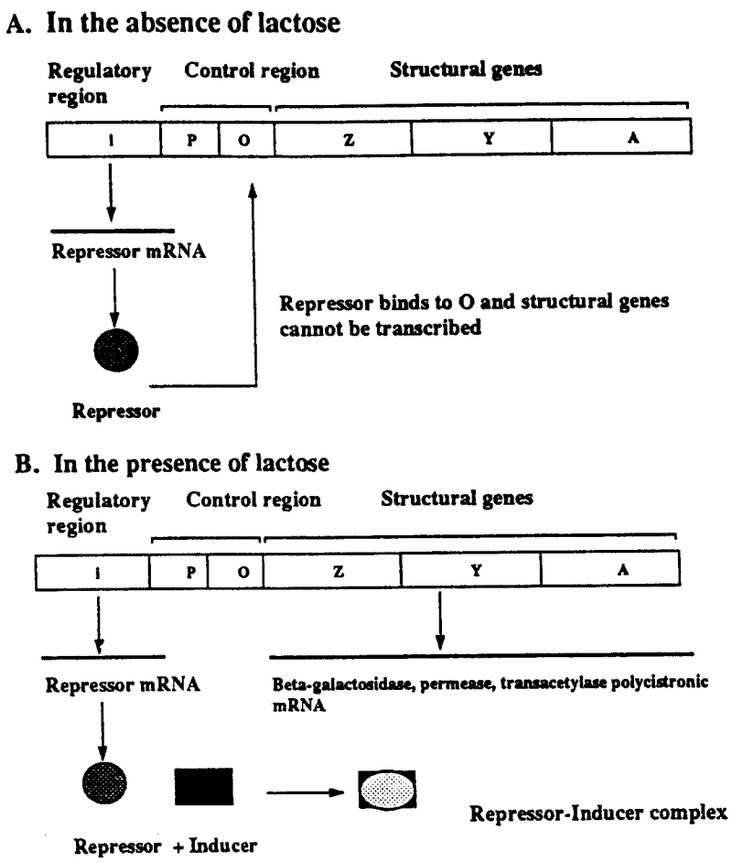

Figure 6-1. Negative control of the lac operon.

Genetic Engineering

Genetic engineering, also known as recombinant DNA technology, provides a means of analyzing and altering genes and proteins. Additionally, this technology can provide a source of specific proteins in almost unlimited quantities, and has many applications in clinical medicine. Genetic engineering depends on having an array of tools for analyzing genes and proteins, including enzymes that can cut, join, and replicate genes.

TOOLS USED IN RECOMBINANT DNA TECHNOLOGY

A. **Enzymes that modify nucleic acids.** Many enzymes that alter the structure of nucleic acids are used in genetic engineering.

1. **Nucleases** constitute a family of enzymes that hydrolyze phosphodiester bonds. They are classified either as ribonucleases or deoxyribonucleases, depending on whether they hydrolyze RNA or DNA. Both ribonucleases and deoxyribonucleases can be further classified as endonucleases (if they cleave internal phosphodiester bonds) or as exonucleases (if they cleave terminal phosphodiester bonds, either at the 5′ or 3′ end of the chain).
2. **Restriction endonucleases** are enzymes that recognize specific double-stranded sequences in DNA and cleave the DNA at or near the recognition or "restriction" site. These enzymes are powerful tools in recombinant DNA technology, allowing specific genes to be excised from the genome. Many restriction endonucleases have been isolated from bacterial sources. **Isochizomers** are restriction endonucleases from two different sources that recognize the same DNA sequence. Most restriction nucleases recognize sites consisting of four to eight base pairs. The sequences in the two strands of the recognition site

are **palindromes**, having the same 5′ to 3′ sequence in both strands. Two examples of restriction endonucleases are shown in Figure 7-1.

NOTE

The location of restriction sites along a DNA helix depends upon its nucleotide sequence:

- *Use of a restriction enzyme on multiple samples of the same sequence will always generate the same pattern of fragment sizes.*
- *A mutation or difference in the DNA sequence can change the presence or absence of a restriction site, thereby changing the sizes of the resulting fragments.*

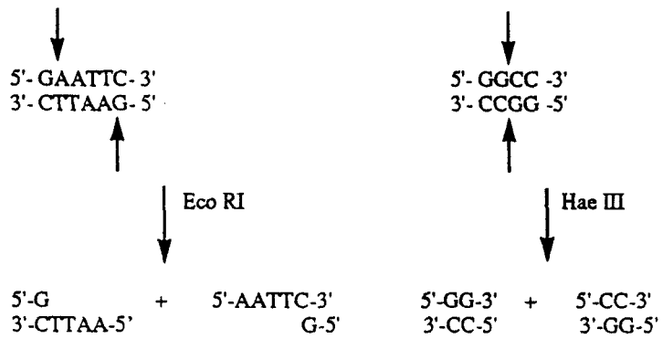

Figure 7-1. Examples of restriction endonucleases.

a. **Eco RI** recognizes a specific six-base-pair sequence in double-stranded DNA and cleaves one site in each strand, as indicated by the arrows, producing staggered cuts that leave short single-stranded tails at the two ends of each fragment. Ends of this type are known as **sticky ends**, since each tail can form complementary base pairs with the tail of any other fragment produced by Eco RI.

b. **Hae III** recognizes a specific four-base-pair sequence in double-stranded DNA and cleaves both strands at positions opposite one another, producing blunt ends with no unpaired tails.

3. **Reverse transcriptase** is an enzyme found in retroviruses whose genome is composed of RNA rather than DNA. This enzyme is an RNA-dependent DNA polymerase that can synthesize a single strand of DNA using a complementary RNA strand as a template. It is used in recombinant DNA technology to construct synthetic DNA molecules known as cDNA (complementary DNA) by copying the corresponding mRNA.

4. **DNA and RNA polymerases** are used to synthesize a particular DNA or RNA, respectively. Unlike RNA polymerase, DNA polymerase requires a primer (a 3′-OH group at the end of an oligonucleotide chain) to initiate synthesis.

5. **DNA ligase** forms a phosphodiester bond between two DNA fragments. This is an essential step in creating recombinant DNA molecules.

6. **S1 nuclease** acts exclusively on single-stranded polynucleotides or single-stranded regions of double-stranded polynucleotides. Double-stranded regions are resistant to its action.
7. **Terminal deoxynucleotidyl transferase** catalyzes the addition of deoxynucleotides to the 3'-OH end of the DNA molecule. It does not require a template (Figure 7-2). Most restriction endonucleases produce complementary "sticky" ends. However, DNA fragments that have "blunt" ends can be joined by using terminal deoxynucleotide transferase to add complementary homopolymer tails. Thus, two molecules can be joined if poly(dA) tails are put on one molecule and poly(dT) tails are put on a second molecule.

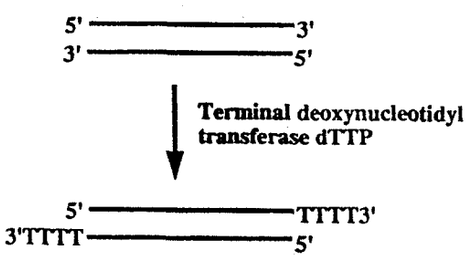

Figure 7-2. Terminal deoxynucleotide transferase reaction.

8. **Alkaline phosphatase** removes the 5' phosphate group from either single-stranded or double-stranded DNA or RNA to give a 5'-OH group.
9. **Polynucleotide kinase** catalyzes the transfer of the terminal phosphate group of ATP to a 5'-OH group in either DNA or RNA. The physiological function of this enzyme is unknown. However, it is useful in radiolabeling nucleic acids, a procedure used in determining the base sequence of polynucleotides. Since naturally occurring nucleic acids usually contain 5'-phosphate rather than 5'-OH, labeling with polynucleotides can be accomplished only after the 5'-phosphate group is removed with alkaline phosphatase.
10. Eco RI methylase catalyzes the methylation of the adenine (A) residue in the Eco RI recognition sequence, thereby protecting the DNA sequence from cleavage by Eco RI.

B. **Oligonucleotides.** Short DNA molecules, usually 16 to 40 base pairs, can be chemically synthesized from nucleotide building blocks. Two specific uses of these oligonucleotides are discussed below.

IN A NUTSHELL

To radioactively label the 5' end of a nucleic acid:

- *Remove existing 5' phosphate with alkaline phosphatase*
- *Use radioactive ATP and polynucleotide kinase to replace it with radioactive phosphate*