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Bacterial Infections

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In Osler's time, bacterial pneumonia was a dreaded event, so important that he borrowed John Bunyan's characterization of tuberculosis and anointed the pneumococcus, as the prime pathogen, "Captain of the men of death."[1] One hundred years later much has changed, but much remains the same. Pneumonia is now the sixth most common cause of death and the most common lethal infection in the United States. Hospital-acquired pneumonia is now the second most common nosocomial infection.[2] It was documented as a complication in 0.6% of patients in a national surveillance study,[3] and has been reported in as many as 20% of patients in critical care units.[4] Furthermore, it is the leading cause of death among nosocomial infections.[5] Leu and colleagues[6] were able to associate one third of the mortality in patients with nosocomial pneumonia to the infection itself. The increase in hospital stay, which averaged 7 days, was statistically significant. It has been estimated that nosocomial pneumonia produces costs in excess of $500 million each year in the United States, largely related to the increased length of hospital stay.

For many years it appeared that the problem of infection in our society had been solved. The discovery and then commercialization of penicillin opened a new era of optimism, which was bolstered by discovery of the macrolides, such as erythromycin, and the aminoglycosides, such as streptomycin. It was not long, however, before the seemingly endless resilience and resourcefulness of bacterial pathogens became manifest. The "Cold War" between medicinal chemists and bacteria began in earnest in the 1960s with the emergence of *Staphylococcus aureus* that were resistant to penicillin and the increasing prominence of gram-negative bacilli as serious pathogens. The war has continued unabated, shifting battlefields as immunosuppressive therapies have been developed and patients survive previously fatal primary diseases. The battle has waxed and waned as new therapeutic strategies have been developed and new mechanisms of resistance have been elucidated. At times the front has encroached on virgin territory, as new infectious agents have been discovered. Legionnaires' disease taught us, in the mid-1970s, that we still had a lot to learn about the enemy. In the 1980s, gram-positive bacteria reasserted their importance, as methicillin-resistant staphylococci and enterococci joined their gram-negative counterparts.[7] It seems we take one step backward for every two forward in our war against bacterial pneumonia. The "Men of Death" still stalk our hospital wards and lurk in our streets for the weak, the unprepared, and the iatrogenically compromised.

Definition and Classification

> Pneumonia (πνεμμονια, of old, meant a disease of the lungs), *Peripneumo'nia*, ... *Pleumo'nia*, ... *Pneumoni'tis*, ... *Pulmonitis, Pulmo'nia*, ... *Lung fever* (vulgarly).... The chief symptoms of pneumonia are: pyrexia, accompanied by pain, sometimes obtuse, at others pungent, in some part of the thorax; pulse more or less quick and hard, according to the violence and extent of the local disorder; pain, aggravated by the cough, which, with dyspnoea, exists throughout the disease.... When the inflammation, instead of going off by resolution, passes on to suppuration, rigors are experienced.... Pneumonia may, also, terminate by gangrene,—but this rarely happens,—by induration and by hepatization.
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> —Robley Dunglison, *A Dictionary of Medical Science*, 1874[8]

Infections of the lower respiratory tract are defined by their anatomic location: larynx, trachea, bronchi, bronchioles, and distal air spaces. Infectious disease of the distal air spaces (respiratory bronchioles, alveolar ducts, and alveoli) is commonly described as pneumonia, but the usage is not always precise and occasionally the generic term *pneumonitis* is used synonymously. Some of the common etiologic agents are capable of producing infections of the trachea, bronchi, and air spaces, but the infections may occur independently as well as concurrently.

TABLE 8.1. Classification schemes for bacterial pneumonia

Category	Variables
Pathogenesis	Exogenous vs. endogenous
	Inhalation vs. aspiration vs. bacteremia
	Primary vs. secondary
Epidemiology	Community acquired vs. nosocomial
Anatomic distribution	Focal (lobular) vs. lobar
	Diffuse vs. circumscribed (round)
Time course	Acute vs. chronic
Etiologic agent	Various

This chapter discusses bacterial pneumonia in adult patients. Certain other bacteria are discussed elsewhere—*Mycobacterium* sp. in Chapter 9, and *Chlamydia* sp. and *Mycoplasma* sp. in Chapter 12.

Pneumonia may be classified in many ways, as demonstrated in Table 8.1. This chapter discusses the pathogenesis, epidemiology, macroscopic distribution, and inflammatory component/time course, and reviews in detail the pathologic processes produced by individual bacteria or bacterial groups.

Pathogenesis of Bacterial Pneumonia

The pathogenesis of bacterial pneumonia begins with the introduction of organisms into the airways. The relative sterility of the lower respiratory tract depends on the filtering action of the nasopharyngeal mucosa, the integrity of the epiglottic barrier, and, finally, the adequacy of pulmonary defense mechanisms (see Chapter 3). Welsh and Mason[9] have reviewed the specific and nonspecific pulmonary defenses, and the review of Green and colleagues[10] also remains a useful general reference.

Intrusions of bacteria into the lower respiratory tract may come from either exogenous or endogenous sources. The few instances in which the source of the inoculum is exogenous are paradoxically less challenging than the more common endogenous infections, because the external source can be identified and controlled. For example, many hospitals have been able virtually to banish *Legionella* pneumonia from their institutions by eliminating the bacteria from the aquatic environment, whether it be in potable water, in adjacent air handling equipment, or both.[11–13] Immunization has been the primary stratagem for control of endogenous pathogens such as *Streptococcus pneumoniae*.[14,15] A classification of bacterial pneumonia by source of the inoculum is shown in Table 8.2

TABLE 8.2. Characterization of bacterial pneumonia by infectious source

Endogenous	Exogenous
Streptococcus pneumoniae	*Mycobacterium tuberculosis*
Haemophilus influenzae	*Mycobacterium* spp.
Anaerobic bacteria	*Legionella* spp.
Staphylococcus aureus	*Yersinia pestis*
Enteric gram-negative bacilli	*Francisella tularensis*
Pseudomonas spp.	*Bacillus anthracis*
Acinetobacter spp.	
Yersinia pestis	
Francisella tularensis	
Miscellaneous	

Routes of Infection

Exogenous Pneumonia

The most important routes by which bacteria are delivered to the air spaces are inhalation of an aerosol, aspiration of respiratory or gastrointestinal secretions, and bacteremic spread. Exogenous pneumonia is predominantly transmitted by inhalation of an infectious aerosol, as exemplified classically by *Mycobacterium tuberculosis* (see Chapter 9). *Bacillus anthracis* produces a variety of diseases, depending in part on the mechanism of bacterial transmission. Cutaneous anthrax, the most common manifestation of infection, results from direct inoculation of spores from contaminated soil or animal skins[16,17] or from exposure to bacteria in the laboratory.[18,19] The infection remains localized to the skin. The most feared form of anthrax, however, is the overwhelming pneumonia that results from inhalation of aerosolized spores. Pneumonic anthrax is fortunately rare, because aerosols of large proportions are uncommon. Careful epidemiologic investigation may be necessary to establish the source of the aerosol, as occurred when workers in a woolen mill contracted inhalational anthrax,[20] after the accident at a biologic warfare facility in Russia,[21] or after the intentional exposures in the U.S. more recently.[22] Analogous situations are found in plague and tularemia, which usually infect the lungs as a by-product of bacteremic disease but may produce primary respiratory infection after inhalation of aerosolized bacteria.

The most recent example of an exogenous infection is *Legionella pneumophila* pneumonia, which as noted is almost exclusively associated with aquatic environments. Most investigators accept that an important source of infection is aerosolization of bacteria from cooling towers.[11,13,23] Much of the evidence is derived from epidemiologic studies, buttressed in many cases by molecular analysis of bacterial isolates. A particularly instructive incident ushered in a pair of epidemics in Burlington, Vermont, during the summer of 1980.[24] Two maintenance workers entered an inactive cooling tower on the roof of the building that houses the College of Medicine to prepare it for the summer season. During their work the fan in the tower was activated, producing a sudden blast

of aerosolized water; the men then turned the fan off and continued their work. Within several days both men became sick and one developed a severe pneumonia that required prolonged hospitalization. Shortly thereafter a biphasic epidemic began that was associated epidemiologically with the cooling tower.[13] Further, strains of *L. pneumophila* serogroup 1, which were isolated from the cooling tower water and from infected patients, displayed the same antigenic profile when tested with a panel of monoclonal antibodies.[25]

Cooling towers are not the only sources of *Legionella* aerosols, however. Small epidemics of legionnaires' disease have been traced to the use in nebulizers of drinking water that was contaminated with *Legionella*.[26] Hypothetically many other daily activities are associated with transient aerosols, such as turning on a faucet, flushing a toilet, or taking a shower. Despite the concentration of attention on cooling towers, the source of most cases of sporadic legionnaires' disease is unknown, but probably includes transmission of bacteria from a source in potable water to the patient. It has not been determined whether the mechanism is inhalation of an aerosol, aspiration of potable water, or both. The role of drinking water has been further emphasized, however, by the dramatic demonstration that *Legionella dumoffii* produced sternal wound infections when tap water was used to bathe the incisions of patients who were recovering from cardiac surgery.[27]

Endogenous Pneumonia

The pathogenesis of most cases of endogenous pneumonia includes transmission of bacteria from the upper respiratory or gastrointestinal tracts into the lung. The mechanism is not always clear, but the most likely scenario is that oropharyngeal secretions are aspirated into the lower respiratory tract (Fig. 8.1). Experimental studies of humans have documented regular episodes of subclinical aspiration, which increase in magnitude as the level of consciousness decreases[28] (also see Chapter 5). The interplay among the volume of aspirated material, microbial composition of the inoculum, and adequacy of defense mechanisms then determines the outcome of the event.

Community-acquired and nosocomial pneumonias of endogenous origin are caused by different groups of bacteria, but in each case the bacterial inoculum originates in the upper airway. Factors that determine the nature of the colonizing oropharyngeal flora also indirectly predict the etiologic agents of lower respiratory infection.

The oropharyngeal bacterial flora of normal individuals is predominantly gram positive.[29] The local environment with its narrow range of temperature and pH, flow of salivary secretions, and humoral factors such as immunoglobulin A (IgA) and lysozyme favor a plethora of streptococcal species.[29] *S. pneumoniae* colonizes the oropharynx in as many as 10% to 15% of patients who do not have pneumococcal infections.[30] *S. aureus* is a frequent inhabitant. *Haemophilus influenzae,* especially nontypeable strains that lack capsular polysaccharide, *Neisseria* species, and *Moraxella* (*Branhamella*) *catarrhalis* are regular gram-negative members of the oropharyngeal flora. Even *Streptococcus pyogenes* (group A β-hemolytic *Streptococcus*), often considered a priori a pathogen when isolated from the oropharynx, may be recovered from asymptomatic individuals. Winther and colleagues[31] studied a group of healthy students five times during a single winter season. *S. pneumoniae* was isolated from 12.5% to 25.6% of students, *S. pyogenes* from 3.5% to 8.3%, and *Haemophilus* species from 1.2% to 8.8% during a period of 8 months. Anaerobic bacteria, both gram positive and gram negative, make their home in the crevices of the gums and proliferate when dental hygiene is poor (see also Chapter 5).

FIGURE 8.1. An aspirated Christmas tree fragment removed from the bronchus of a young child. The specimen yielded pure growth of *Streptococcus pneumoniae.* Most episodes of aspiration are subtle and unrecognized by the patient.

Colonization

Once patients are admitted to the hospital, there is a dramatic shift in the colonizing flora of the upper airway from gram positive to gram negative. Enteric gram-negative bacilli and *Pseudomonas* species join or even replace gram-positive bacteria as colonizing flora of the upper airways. The alteration in flora does not occur in physiologically normal subjects who have been hospitalized and correlates best with severity of illness.[32] Gram-negative

bacilli were isolated from pharyngeal cultures in 2% of normal subjects, whether hospitalized or not, from 16% of moderately ill patients, and from 57% of moribund individuals. This shift in colonizing flora is not related to duration of hospitalization and in fact frequently occurs within 24 hours of admission to an intensive care unit (ICU).[33] Cigarette smokers and patients with chronic bronchitis also experience a change in the colonizing flora to gram-negative species.[34]

The importance of colonization in the pathogenesis of pneumonia is emphasized by the prominence of gram-positive bacteria, *H. influenzae,* and anaerobic bacteria in the etiology of community-acquired pneumonia and by the prominence of gram-negative species in nosocomial pneumonia. The anaerobic bacteria most commonly isolated from pulmonary infections include anaerobic streptococci, *Fusobacterium nucleatum,* and black-pigmented genera such as *Porphyromonas* and *Prevotella* (formerly *Bacteroides melaninogenicus* group).[35–37] *Enterococcus* species, which heavily colonize the lower gastrointestinal tract and participate in abdominal infections, are notably infrequent in the oropharynx and infrequently produce bacterial pneumonia.

In one study of nosocomial pneumonia, Johanson and colleagues[33] noted that 22 of 26 patients in an ICU who developed bacterial pneumonia had been colonized previously with gram-negative bacilli. Pneumonia resulted in 3.3% of patients who had not been colonized, but fully 23% of colonized patients developed infections of the air spaces.

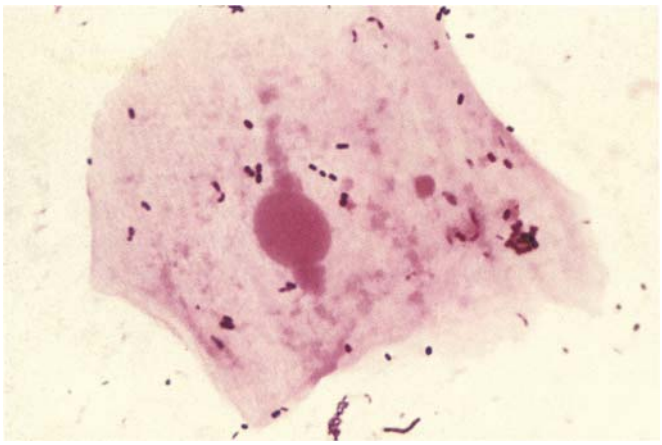


FIGURE 8.2. Gram-positive cocci and bacilli adhere to the surface of a desquamated squamous epithelial cell in an expectorated sputum specimen. In many hospitalized patients, gram-positive organisms are replaced by gram-negative bacilli. (Gram stain.)

Bacterial Adherence

The mechanism by which colonization occurs appears to involve adherence of bacteria to epithelial cells (Fig. 8.2). In a group of 34 patients who required intensive care, 53% were colonized with gram-negative bacilli.[38] The buccal epithelial cells from colonized patients contained more adherent gram-negative bacilli and fewer adherent α-hemolytic streptococci than the cells of noncolonized individuals. Further, incubation of cells in vitro with one species of enteric bacillus inhibited adherence of a second species, suggesting that a specific receptor was responsible for the phenomenon. When buccal squamous epithelial cells from a group of 32 noncolonized patients who were undergoing cardiac surgery were studied in vitro, the frequency of adherence of *Pseudomonas aeruginosa* and three other gram-negative bacilli doubled in half the patients. Eleven of 16 (69%) patients whose buccal cells developed an affinity for gram-negative bacteria in vitro became colonized in vivo.[39] None of the patients who did not develop increased adherence in vitro in the perioperative period became colonized in vivo. Adherence to tracheal columnar epithelial cells may also be important. Todd and colleagues[40] studied the adhesion of *P. aeruginosa* to tracheal cells in a group of 24 ICU patients. Pneumonia was observed in 11 of 12 patients who had elevated bacterial adherence in contrast to only 1 of 12 patients with normal adherence. A prospective study was not performed, however.

The bacterial adhesive factors vary greatly, depending on the species.[41,42] In addition, the interactions between bacterial and host cells is clearly multifaceted, as reviewed by Wilson and colleagues.[43] As suggested by the epidemiologic studies, however, one critical factor appears to be inherent changes in the epithelial cells that permit access to previously hidden receptors. Fibronectin is the prime candidate for the critical cellular factor. Woods and colleagues[44] studied 12 seriously ill patients whose respiratory tract was colonized with *P. aeruginosa,* and a group of noncolonized controls. The sialic acid and fibronectin content of buccal epithelial cells from the seriously ill patients was less than that of controls. When normal cells were treated with neuraminidase to remove sialic acid, there was no change in bacterial adhesion, but trypsinization to remove fibronectin caused increased adherence of *Pseudomonas* to cells from the normal controls. The authors were able to demonstrate decreased cellular fibronectin by immunofluorescence and increased numbers of *Pseudomonas* by Gram stain on the cells of colonized patients and on the trypsinized cells of controls. The factor that strips fibronectin from the surface of cells appears to be protease excreted into the saliva from the salivary glands of colonized patients.[45]

Bacterial Clearance

After inhalation or aspiration of bacteria and other particles, a congeries of defense mechanisms attempts to

eliminate (clear) the particles from the lower respiratory tract. Many experimental studies have documented the variety of interactions that may take place, depending in part on the nature of the infecting organism, the bacterial inoculum, and the state of the infected host. Experimental systems that deliver bacteria to the lungs by aerosol utilize chambers that expose the whole animal[46] or only the snout.[47] These systems most closely simulate inhalation of nebulized or aerosolized bacteria from exogenous sources. Models that employ intranasal or intratracheal inoculation of bacterial suspensions resemble aspiration of endogenous or colonizing microflora.[48]

Bacteria are removed from the lung by two pathways: (1) retrograde up the respiratory tract on the "mucociliary escalator," to be expectorated or swallowed; and (2) into the interstitium.[49] Transport centripetally out of the lung occurs by both cellular and noncellular mechanisms. Free particles are swept along the surface of the ciliated mucosa, following the flow of fluid being removed from the lung.[50] An additional and probably more important mechanism is the removal of cells, especially resident alveolar macrophages, that have phagocytosed ingested bacteria.[51] Clearance of bacteria often follows a biphasic curve, and the ciliary mechanism accounts for the early phase, which occurs in a matter of minutes to hours. *S. pneumoniae* radiolabeled with technetium was cleared from the lung, but did not appear in the hepatic reticuloendothelial system within the first 6 hours after aerosol challenge.[52] Factors that reduce mucociliary clearance, such as influenza virus infection, also reduce the clearance of inhaled particles from the lower respiratory tract.[53] Ultrastructural examination of human upper-respiratory ciliated epithelium from children with respiratory viral infections demonstrated damaged cilia that persisted for 2 to 10 days after the infection.[54]

In contrast to the luminal mechanism, the interstitial transport pathways appear to be oriented radially along the terminal bronchiole and thence to the lymphatics and either the pleural surface or regional lymph nodes.[49] The familiar distribution of carbon in the pleura, peribronchial region, and lymph nodes at postmortem examination reflects this pathway (Fig. 8.3). This second phase of clearance is considerably slower and may last days, weeks, or months. It is, however, crucial for the development of an adaptive immune response, through antigen processing and presentation, which ultimately lead to antibody production and immunologic memory.

When clearance is measured by titration of viable bacteria, a more rapid disappearance of the inoculum is usually observed (Fig. 8.4), representing cellular bactericidal activity as well as physical removal of the particles.[55] Experimental variations occur depending on the design of the aerosol chamber, the nature of the bacterial species, and the placement of animals, but these factors can be controlled. Killing of bacteria by drying and shear forces while the aerosol is being generated cannot be completely eliminated.[56,57]

FIGURE 8.3. Confluent focal (lobular) pneumonia is produced by coalescence of individual lobular lesions. A previously healthy young man, a cigarette smoker, developed acute *Legionella* pneumonia. *Legionella pneumophila* serogroup 1 was isolated in a pure culture from lung tissue. This paper-mounted whole-lung section (Gough technique) demonstrates well the association of inflammatory exudate with pulmonary lobules, because centrilobular regions are marked by intense carbon pigmentation. Lobular lesions are evident in the upper lobe. Consolidation has become confluent in the lower part of the upper lobe and throughout the lower lobe. Such extensive consolidation may be characterized as lobar or sublobar if the focal nature of the process is not recognized.

The interaction between the infecting bacteria and the resident phagocytic cells is a critical factor in the outcome. When most respiratory pathogens are introduced into the air spaces, physical clearance occurs and bactericidal activity is expressed to varying degrees. In the initial encounter with an infectious agent, defense mechanisms are not immunologically specific, and the complement segment of the humoral immune system plays an important role. Some bacterial species, such as *S. aureus,* are readily phagocytosed by resident macrophages. In contrast, encapsulated bacteria, such as *S. pneumoniae* and *H. influenzae* type b, are inherently resistant to phagocytosis in the absence of opsonins. Pneumococci are able to activate the complement cascade, either through the classical pathway in the presence of antibody or through the

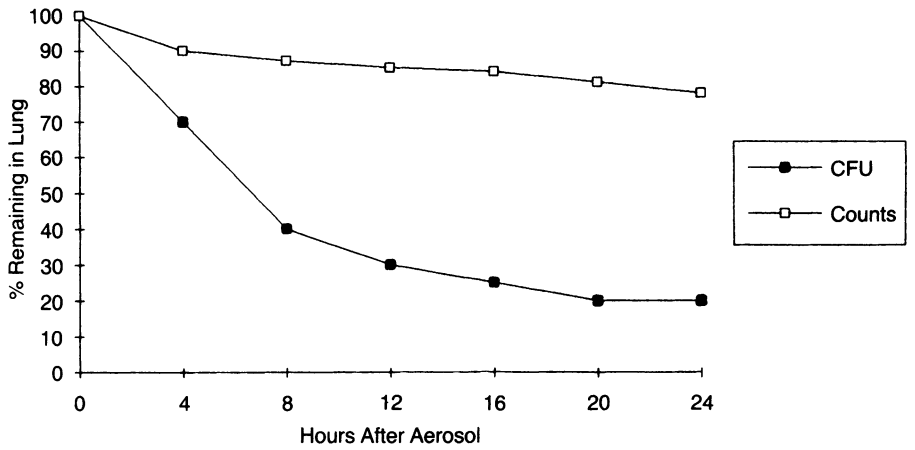


FIGURE 8.4. Clearance of physical bacterial particles and viable organisms from the lung in experimental pneumonia. After an aerosol challenge, physical particles (represented by radioactive counts) are removed slowly over a period of days. Effective clearance of viable bacteria combines physical removal with the antibacterial defenses of the lung. CFU, colony-forming unit.

alternate pathway when antibody is absent.[58] Winkelstein and Tomasz[59] demonstrated that the teichoic acid component of the cell wall is responsible for the activation of the alternate pathway. Phagocytosis of pneumococci by neutrophils is enhanced by complement, antibody, or conserved bacterial products (known as "patterns"), which interact with cellular complement receptors, Fc receptors, and Toll-like receptors respectively.[60,61] It appears that there are serotypic differences among pneumococci in their interaction with the complement system. Coonrod and colleagues[62] reported that type 1 strains were not opsonized by complement, type 3 strains were opsonized partly by the alternative pathway and partly by the classical pathway, and type 25 strains were readily opsonized by the alternate pathway.

The importance of complement is illustrated by the occurrence of overwhelming pneumococcal infection in patients with deficiencies in several complement components.[63] Experimentally, the effect of complement depletion on the clearance of bacteria from the lung depends on the in vitro interactions among complement, cells, and bacteria. Several groups have demonstrated impaired clearance of *S. pneumoniae* and *P. aeruginosa* in animals rendered hypocomplementemic by cobra venom factor.[64,65] In contrast, clearance of *S. aureus* and *Klebsiella pneumoniae* was unaffected by decomplementation.

Nonimmunologic humoral defenses may also be important in the early hours after bacteria are deposited in the lower airways. Coonrod and colleagues[62] demonstrated that encapsulated pneumococci were killed in vitro by incubation with a fraction of rat bronchoalveolar fluid that was rich in surfactant. In addition, LaForce and associates[66] demonstrated that rat alveolar lining material enhanced the antibacterial activity of macrophages. Other investigators, using concentrated human alveolar fluid, have been unable to demonstrate any antibacterial activity.[67] Further detailed analysis of surfactant components has revealed a wide array of compounds with antimicrobial properties. These include a subset of C-type lectins, called collectins, such as surfactant proteins A and D; acute phase reactants; and soluble proteins with pore-forming capabilities, referred to as defensins.[68–70] In addition to producing defensins, type II alveolar epithelial cells also have been found to upregulate surface expression of molecules of the integrin family, which are typically produced by activated endothelium.[71] O'Brien and colleagues[72] corroborated these data in a murine model of pneumonia by demonstrating that expression of intercellular adhesion molecule-1 (ICAM-1) by alveolar pneumocytes markedly enhances intraalveolar phagocytosis of *K. pneumoniae* by macrophages and neutrophils. They were able to show also that ICAM-1 knockout mice had significantly higher mortality rates after *Klebsiella* infection than did control mice in the absence of any variation in recruitment of neutrophils into the lung. Such nonimmunoglobulin factors as complement and alveolar lining material may explain the early observations in experimental pneumococcal pneumonia that limitation of the expanding pneumonic lesion and phagocytosis of bacteria occurred so soon after infection that specific antibody could not be responsible for the effective host defense.[73]

Facultative intracellular pathogens, such as *L. pneumophila* or *M. tuberculosis,* actually proliferate in the resident alveolar macrophages after phagocytosis so that only the relatively small component of physical clearance is operative.[74] In this situation the best strategy from the point of view of host defenses is to keep bacteria out of macrophages; opsonins may actually be undesirable. In vitro, at least, the addition of specific antibody opsonin to

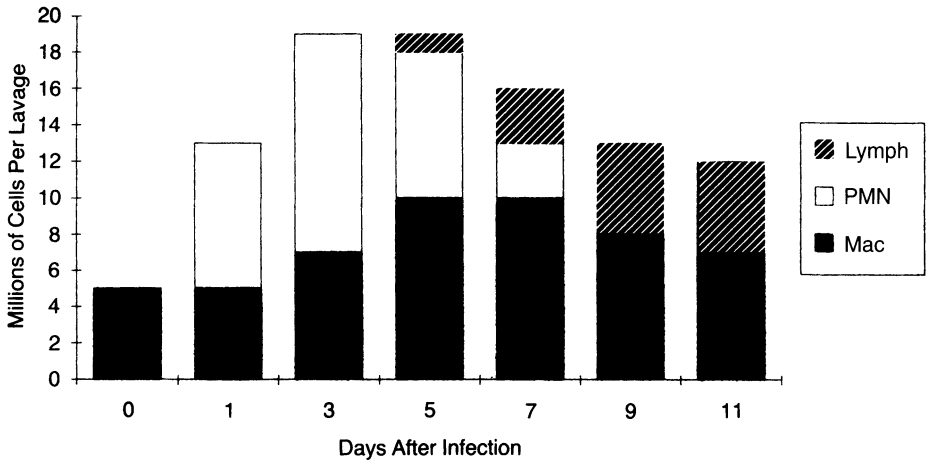


FIGURE 8.5. Cell populations in air spaces in bacterial pneumonia. The predominant cell in air spaces at the onset of infection is the alveolar macrophage (Mac). Recruited polymorphonuclear neutrophils (PMNs) quickly become predominant cell type and are removed as the acute infection is contained. Recruited monocytes then increase numbers of macrophages and are cleared more slowly. Recruited lymphocytes (Lymph), appearing approximately 5 to 7 days after infection represent the cellular phase of the immune response.

cultures of *L. pneumophila* and human alveolar macrophages promotes adherence of bacteria and does not inhibit intracellular multiplication.[75]

Inflammatory Responses

The resident alveolar macrophages are the critical first line of cellular defenses, but recruited polymorphonuclear neutrophils and circulating monocytes are essential backup forces (Fig. 8.5). Once the inflammatory process is initiated, a complex set of interactions among cells, bacteria, and plasma proteins sets in motion a series of events that is perpetuated by a continuing network of chemotactic stimuli.[76] The precise sequence of events depends in part on the virulence of the infecting bacterium and the quantity of organisms in the challenge. Onofrio and colleagues[77] studied the interactions of inflammatory cells and graded quantities of *S. aureus* that had been introduced into the lungs of mice. If the inoculum is small and the resident macrophages are capable of engulfing and killing the bacteria, there is no need for additional cellular recruitment and chemotactic factors are not generated. With intermediate inocula an active recruitment of polymorphonuclear neutrophils ensues, after which bacterial growth is checked. If the inocula are sufficiently high, neutrophils are recruited but the combined cellular response is inadequate to control the growth of bacteria.

The inflammatory sequence of resident alveolar macrophage, recruited polymorphonuclear neutrophil, and recruited monocyte-macrophage can be generalized from several experimental models.[74,77–79] Although there may be variation in the propensity for recruitment of polymorphonuclear neutrophils, the standard response is an influx of neutrophils within a few to 24 hours after infection. Pierce and associates[79] found that two enteric gram-negative bacilli, *Escherichia coli* and *K. pneumoniae,* elicited a greater neutrophilic response in the early hours after aerosol infection of mice than did *S. aureus* or water. Vial and colleagues[80] demonstrated a dose dependence for *S. pneumoniae.* Both the recruitment of neutrophils into the air spaces and the chemoattractant ability of bronchoalveolar lavage fluid in vitro were directly related to the bacterial inoculum (log neutrophils recruited = 0.751 log *Pneumococcus* + 0.52; $r = .77; p < .005$).

The stimulus for the neutrophilic influx is multifactorial. An important mechanism is the activation of the complement cascade through either the classical, mannose-binding lectin (MBL), or alternate pathways. Shaw and associates[81] instilled low molecular weight fragments of C5 into the trachea of rabbits and reproduced the full spectrum of the inflammatory response. Damage to endothelial and epithelial cells, extravasation of erythrocytes with fibrin clumps, and degranulating polymorphonuclear neutrophils were evident ultrastructurally. The process could be blocked by absorption of complement fragments with antibody to homogeneous human C5a. Larsen and colleagues[82] have suggested that C5a des arg, a metabolite of C5a produced in vivo, is actually responsible for the phlogistic responses in the lung. Coonrod and Rylko-Bauer[83] have documented depressed levels of complement components that participate in the alternate pathway during the early phases of pneumococcal pneumonia, suggesting that complement activation is a part of the pathophysiologic response in human disease.

Chemotactic stimuli for neutrophils are also derived from resident alveolar macrophages and epithelial cells. When particles such as Sepharose beads or bacteria are phagocytosed by alveolar macrophages in vitro, the stimulated macrophages release a low molecular weight chemotactic factor that is distinct from C5a. Incubation of the particles with serum augments the chemotactic response and causes the fixation of C3b onto the particle surface.[84] A variety of such soluble factors, having either inherent chemoattractant properties or the capacity to activate crucial components of the inflammatory pathway, have been elucidated during the past decade. These include a multitude of cytokines (interleukin-1 [IL-1], tumor necrosis factor-α [TNF-α], interferon-γ [IFN-γ], granulocyte colony-stimulating factor [G-CSF]), prostaglandin derivatives (leukotriene B_4 [LTB_4]) as well as chemokines (IL-8, macrophage inflammatory protein [MIP-2]).[70,84,85]

The appearance of polymorphonuclear neutrophils in the air spaces coincides with the development of leaky capillaries and transudation of immunoglobulin, albumin, and other serum factors.[74] Ultrastructurally, there is damage to endothelial cells and movement of neutrophils between the cells, through the basement membrane, and into the interstitium. Damage to epithelial lining cells and movement of neutrophils through the gaps into the alveoli ensues if the chemotactic response originates in the air space (Fig. 8.6).[81,86] Experimental damage to the pulmonary vascular endothelium is mediated by polymorphonuclear neutrophils and is blocked by depletion of circulating neutrophils, whether the process initiates within the vascular system[87,88] or within the alveoli.[81] In the trachea, neutrophils migrate through the mucosa serially, not randomly between epithelial cells.[89]

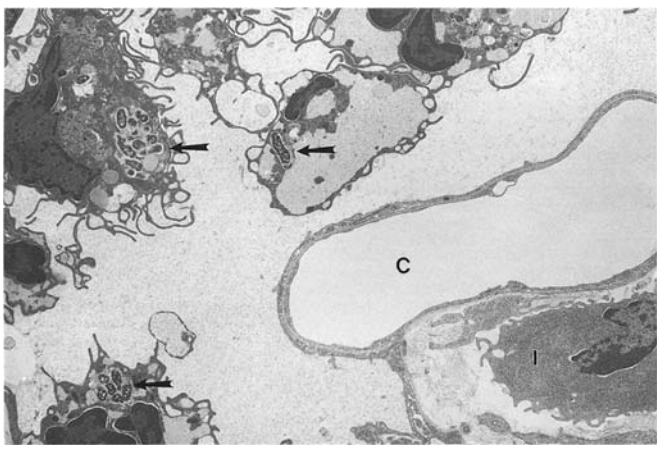


FIGURE 8.6. Electron micrograph of a guinea pig lung infected with serogroup 1 *Legionella pneumophila.* Inflammatory exudate has accumulated in the edematous air space; phagocytosed bacteria are present in phagosomes (arrows). Lungs were fixed by vascular perfusion so that the capillary space has been cleared C.; note the monocyte in the expanded interstitium (I). Glutaraldehyde-fixed osmicated lung stained with uranyl acetate.

Accumulation of neutrophils in the air spaces begins within hours of infection and continues for as long as 3 days. The next phase in the inflammatory process is the recruitment of circulating peripheral blood monocytes, which begins within 1 to 2 days after infection and continues for days to weeks, depending on the nature of the stimulus. The origin of these cells in the bone marrow has been established by radiolabeling and by irradiation of bone marrow in experimental animals.[90] By the time the monocytes traverse the interstitium and reach the air spaces, they are mature macrophages. These recruited macrophages differ immunochemically and functionally from the resident alveolar macrophages. They show evidence of having ingested neutrophilic enzymes, and their anticoagulant and fibrinolytic activity is increased to maximize removal of fibrin.[91] Completing the cycle of inflammatory events, this recruitment of monocyte-macrophages is elicited by chemotactic factors produced by polymorphonuclear neutrophils. A complement stimulus for inflammatory cells does not result in macrophage recruitment if animals have been first rendered neutropenic.[92]

The final inflammatory cell component is the lymphocyte, which appears in the air spaces 5 to 7 days after infection.[74] The lymphocyte is not usually considered a critical mediator of the inflammatory pneumonic process, possibly due to its late arrival at the scene. Yet its integral role in orchestrating the adaptive arm of the immune response, which takes place largely in nearby lymphoid organs—outside of the pulmonary parenchyma—is worth considering. Specific mucosal immunity in the form of secreted and circulating antibodies as well as cell-mediated destruction of virulent intracellular organisms are contingent upon the viability and competence of certain lymphocyte populations.[9] There is experimental evidence that these immunologically specific cells may differ depending on the nature of the stimulus. Shennib and colleagues[93] noted that subsets of lymphocytes had distinctive characteristics when the injury was lung allotransplantation or *P. aeruginosa* pneumonia in experimentally treated dogs.

The lung has a limited repertoire of responses to acute injury. When that injury is sufficiently severe, the initial specific lesions become rapidly overshadowed by a final common response and diffuse alveolar damage (DAD) results.[94] The concept of DAD is critically important for understanding many types of infectious and inflammatory lung diseases. It provides a template for the sequential way the lung responds to a brief injury and for how repair is accomplished in an organ that is mostly composed of air (see also Chapter 4).

When type I epithelial lining cells of the alveoli are damaged diffusely, they lose their barrier function, leading

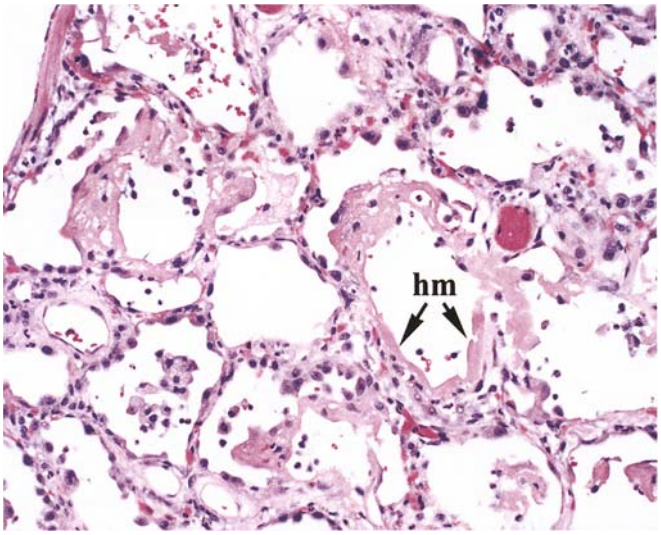


FIGURE 8.7. Hyaline membranes in diffuse alveolar damage (DAD). The early exudative phase (days 1 to 3) of diffuse alveolar is characterized by edema, and serum exudation into the alveolar spaces. Proteins coalesce to form brightly eosinophilic membranes (hm) on the inside surface of the alveoli.

to flow of interstitial water into the air spaces.[94] Alveolar edema occurs. Serum proteins and tissue debris accumulate and coalesce to form *hyaline membranes* (Fig. 8.7). These microscopic events correlate with the "red hepatization" phase of lobar pneumonias as described by Laennec (see below). Within a few days, macrophages begin to accumulate in the air spaces and begin to clear the cellular debris. Simultaneously, interstitial fibroblast-like cells proliferate and migrate into the air spaces. These migrating fibroblasts become *myofibroblasts* capable of exerting tractional forces on surrounding cells and structures and eventually secrete collagen matrix, providing the principal manifestation of the "organizing" or "proliferative" phase (Fig. 8.8) of DAD.[95] It corresponds to Laennec's "gray hepatization" phase of lobar pneumonia (see below). In time, myofibroblasts and their associated matrix are reabsorbed into the alveolar wall by cellular contraction and eventual apoptosis. Variable collagen deposition occurs, depending on the extent, severity, and duration of the initial insult. Thus, after a limited insult, such as experimental oxygen toxicity, there is only slight residual tissue alteration, consistent with a return to normal or near-normal lung function. In fact, minimal functional deficits have been demonstrated in follow-up studies of patients who have recovered from acute respiratory distress syndrome (ARDS).[96]

Effect of Immunity on Defense Mechanisms

There are relatively few clinical or experimental data on the effectiveness of the immune response in preventing repeat episodes of pneumonia. Second episodes of *L. pneumophila* serogroup 1 pneumonia have not been documented definitively. Experimental studies with this pulmonary pathogen have documented solid protective immunity after a primary infection.[97] Protective immunity to *L. pneumophila* can be produced by immunization with the major secretory protein of the bacterium.[98] The immune response to *L. pneumophila* is both humoral and cellular. In vitro studies document the association of this pathogen with macrophages, the inability of specific antibody to prevent intracellular growth, and the ability of activated macrophages to inhibit growth within the cells.[99,100] These characteristics of the bacterium suggest that cellular immunity represents the primary defense in vivo also.

In contrast, *H. influenzae* pneumonia that is associated with subsequent bacteremia is usually caused by strains that possess the type B capsular polysaccharide. The recent introduction of an immunogenic vaccine to the capsular polysaccharide has been associated with a dramatic reduction in the incidence of invasive *H. influenzae* type B disease.[101]

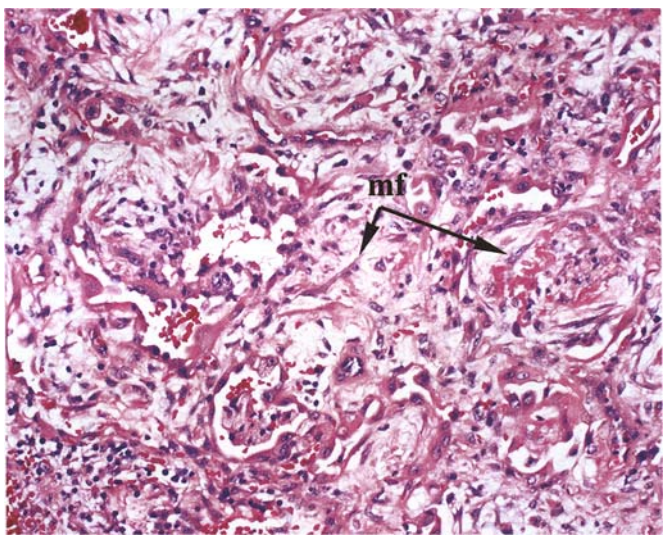


FIGURE 8.8. Proliferative phase of DAD. Several days after the onset of diffuse alveolar damage, repair ensues with the migration of contractile fibroblasts (*myofibroblasts*) into the alveolar spaces (mf). These reparative cells serve a number of functions in the reparative process (tissue contraction, collagen secretion). When the injury event is complete and fails to disrupt the basement membranes, these cells contract into the interstitium where they presumably undergo programmed cell death (apoptosis). The origin of these myofibroblasts is not completely known. Some hypothesize that they are derived from mesenchymal stem cells resident in the alveolar interstitium. New evidence suggests that they derive from circulating cells (monocytes or stem cells) that differentiate in situ. (From Epperly MW, Guo H, Gretton JE, Greenberger JS. Bone marrow origin of myofibroblasts in irradiation pulmonary fibrosis. Am J Respir Cell Mol Biol 2003;29(2):213–224.)

Similarly, a primary therapy for pneumococcal pneumonia before the development of effective antibiotics was immunotherapy with type-specific antibody. In classic studies performed by W. Barry Wood,[73] administration of antibody to experimental rats that had been infected with *S. pneumoniae* dramatically limited the spread of the inflammatory process, cleared the bloodstream of bacteria, and prevented the extension of early pleurisy. Musher and colleagues[102] demonstrated a quantitative relationship between the amount of anticapsular antibody to *S. pneumoniae* type 4 and protection from infection in experimental mice. In recent years several polysaccharide-protein conjugate immunogens (now included in both *S. pneumoniae* and *H. influenzae* vaccines) have proven remarkably effective, in the process elucidating a variety of adaptive immune mechanisms.[103–106]

The large variety of immunotypes in many bacterial species makes assessment of protective immunity difficult. Recurrent infections with nonencapsulated strains of *H. influenzae* in patients with chronic obstructive pulmonary disease suggest, but do not prove, that immunity is incomplete.

Some of the effects of prior infection or immunization may be mediated by the mechanisms discussed. For example, mice that had been immunized with *P. aeruginosa* cleared bacteria from the lung more effectively than their nonimmunized counterparts.[107] Some of the effects of immunization may be immunologically nonspecific but instead be caused by activation of the cellular phagocytic defenses of the lung. LaForce and colleagues[108] demonstrated enhanced intrapulmonary bactericidal activity against aerosolized *Serratia marcescens, E. coli,* and *S. aureus* in mice that had been experimentally immunized with the Re595 strain of *Salmonella minnesota* 2 weeks previously.

Secondary Pneumonia

In the scenarios outlined previously, pneumonia is the primary event. Bacteria may also reach the lung through the bloodstream after initiating a distant infection. The capillary bed of the lungs is one of the important filters in the circulatory system, providing an efficient trap for circulating bacteria or infected thromboemboli. The roster of microbes that produce secondary pneumonia includes important pathogens such as *S. aureus,* enteric gram-negative bacilli, *Salmonella typhi, Francisella tularensis,* and *Yersinia pestis.*

When the bacterial inoculum is delivered to the lung through the bloodstream, the defense mechanisms are different and the interactions vary correspondingly. Harrow and colleagues[109] studied the effect of systemic inoculation of *S. aureus* and *Proteus mirabilis* in mice. They found that larger quantities of *S. aureus* lodged in the lung and that this bacterial species was killed by pulmonary defenses less efficiently than was the gram-negative organism. These experimental studies may have their counterpart in the frequency with which *S. aureus* causes nosocomial pneumonia.

Epidemiology

Community-Acquired Versus Nosocomial Pneumonia

The geographic site at which the pneumonia was acquired is of more than academic interest. As has been indicated, the colonizing oropharyngeal flora vary by age and hospitalization status. Knowledge of the most likely etiologic agents directs the selection of the most appropriate antibiotics. The patient-related risk factors also differ in community-acquired and nosocomial infections. A summary of etiologic agents for community-acquired and nosocomial pneumonia is presented in Tables 8.3 and 8.4. There is surprising agreement among these diverse studies, which were performed in various populations using nonstandardized methods. It is particularly important to note that the results are influenced by the diagnostic methods used, by the types of agents sought, and by the criteria for documenting etiologic agents. As suggested by the data on normal flora and colonization of the upper airways, the predominant pathogens in community-acquired pneumonia are either members of the normal oropharyngeal flora or are exogenously acquired organisms such as *Legionella* species, *Mycoplasma pneumoniae,* and viruses. *S. pneumoniae* no longer occupies the overwhelmingly predominant position of earlier decades, but it still accounts for a substantial minority of infections. The switch to gram-negative pathogens, including *P. aeruginosa,* and traditional hospital pathogens, such as *S. aureus,* is evident in the causes of nosocomial pneumonia.[110]

It is important to document local conditions, because both pathogens and their susceptibilities to antimicrobial agents may vary greatly. The presence of potential pathogens in the local environment and the existence of effective means for transmission of microbes to patients accounts for some variation, as demonstrated by epidemics of *Legionella*[111] and *Aspergillus*[112] infection. Methicillin-resistant *S. aureus,* enteric gram-negative bacilli, and non–glucose-fermenting gram-negative bacilli such as species of *Pseudomonas* and *Acinetobacter* may cause local problems because of patterns of practice, including use of antibiotics, and because of susceptibility of particular patient populations.[113] Molecular diagnostic tools may be useful for dissecting the epidemiology of the infections.[114–116] Special epidemiologic intervention, such as isolation of patients, reinforcement of hand-washing

TABLE 8.3. Etiology of community-acquired pneumonia

Reference	Holmberg[812]	Karalus et al.[813]	Woodhead et al.[814]	Lim et al.[815]	Fang et al.[816]	Marrie et al.[817]	Torres et al.[818]	Fine et al.[819]	Bohte et al.[820]	Bates et al.[821]	Pachon et al.[822]	Marston et al.[157]
Country	Sweden	Britain	Britain	Australia	United States	Canada	Spain	United States	The Netherlands	United States	Spain	United States
Number of cases	147	92	236	106	359	719	92	347	334	154	67	2776
Age (years)	71[a]	56[b]	15–79[c]	60[b]	62[b]	63[b]	53[b]	62[b]	65[a]	64[b]	57[b]	
Streptococcus pneumoniae	46.9%	33%	36%	42%	15.3%	8.5%	15%	16%	27%	5.2%	18%	12.6%
Haemophilus influenzae	9.5%	2.1%	10%	9%	10.9%	3.8%		11%	7.8%	1.3%	3.0%	6.6%
Legionella spp.	2.7%	4.2%	0.5%	3%	6.7%	1.9%	14%	6.6%	2.3%	8.4%	10%	3.0%[d]
Mycoplasma pneumoniae	5.4%	18%	1%	8%	2.0%	5.6%	6%	2.0%	5.7%	1.9%		32.5%[d]
Moraxella (*Branhamella*) *catarrhalis*	2.0%					0.2%			1.5%			0.76%
Aerobic gram-negative bacilli		5%	1.5%	8%	5.9%	4.4%	9.8%	5.2%	3.6%	5.2%	12%	4.5%
Staphylococcus aureus		3.3%	1%	3%	3.3%	4.0%		3.1%	1.2%	4.5%	1.5%	3.4%
Influenza A	9.5%	8%	6%	4%		5.6%			4.2%	3.9%		7.4%[d]
Chlamydia psittaci	1.4%	2%	1%	5%								
Chlamydia pneumoniae					6.1%			5.4%	2.7%	5.2%		8.9%[d]
Aspiration/postobstructive pneumonia					8.6%	9.0%	4%	9.2%				
Mixed infections	10.3	11%	20%	18%	2.8%	10.3%			10%	6.5%	3.0%	1.8%[d]
Unidentified/miscellaneous	22.6%	24.4%	43%	18%	49.8%	49.8%	48%	46%	45%	49%	50%	

[a]Median.
[b]Mean.
[c]Range.
[d]n < 2776 (subset analysis).

TABLE 8.4. Etiology of nosocomial pneumonia

Reference	Torres et al.[823]	Fagon et al.[824]	Bartlett et al.[35]	Bryan and Reynolds[825]	Quoted in Scheld and Mandel[2]	Rello et al.[292]	Rouby et al.[185]	Rello et al.[826]
Year	1989	1989	1986	1984	1984	1991	1992	1991
Country	Spain	France	United States[c]	United States[d]	United States	Spain[b]	France[a,e]	Spain
Number of cases	78	52	159	172	—	68	43	54
Age (years)	54.5[f]	65[f]	68[g]	61–70[h]	—	—	57[c]	50[f]
Streptococcus pneumoniae		3%	44%	17%		4%		5%
Staphylococcus aureus	3%	33%	10%	27%	12.9%	22%	29%	28%
Enteric gram-negative bacilli	3%	16%	14%	33%	37.4%	8%	17%	15%
Pseudomonas spp.	6%	23%	3%	9%	16.9%	21%	33%	26%
Acinetobacter spp.	12%	11%				3%	7.1%	4%
Moraxella (Branhamella) catarrhalis		8%			2%			2%
Haemophilus spp.		8%	3%			19%		24%
Legionella spp.	3%	1%						
Mixed infections	16%	40%	—	12%	—	28%	29%	24%[e]
Miscellaneous unidentified	76%	—	26%	14%	32.8%	21%	35%	13%

[a]Patients undergoing mechanical ventilation.
[b]Case definition required isolation of $>10^3$ colony-forming units of bacteria from protected catheter bronchoscopy.
[c]Etiology defined by isolation in pure culture.
[d]Etiology defined by positive blood culture.
[e]Mixed infections included within individual categories.
[f]Mean.
[g]Median.
[h]Mode.

technique, and restriction of antibiotic use, may be necessary to control the spread of drug-resistant pathogens.[117]

Risk Factors for Development of Bacterial Pneumonia

The risk factors for development of pneumonia are summarized in Table 8.5. The most rigorous studies are prospective, case-controlled epidemiologic analyses with sophisticated statistical evaluation. Those studies that utilize multivariate analysis and logistical regression are particularly instructive because they help to eliminate variables that are not independent risk factors. The quality of the information, not surprisingly, is considerably higher for nosocomial infection, because the population of patients at risk can be defined more precisely and appropriate controls identified more readily than can be done in community-acquired pneumonia. It is nevertheless obvious that many of the risk factors are similar.

In many instances the documented risk factors reflect variables that are operative in the pathogenesis of pneumonia. Conditions that predispose patients to aspiration, such as depressed consciousness from neurologic disease, anesthesia, or prolonged intubation, are prominent on the list. It is important to recognize that intubation does not prevent aspiration; in fact, the violation of laryngeal integrity by a foreign body may foster episodes of aspiration. It is well established that the tubing of modern ventilators, equipped with cascade humidification systems, become contaminated by flora that originated in the patient.[118] These tubes provide a source for a bacterial inoculum that can be dumped back into the patient's lower respiratory tract as the patient is moved about. This mechanism may be responsible for the surprising finding that the increased manipulation caused by daily tubing changes actually increases the risk of pneumonia.[119] A recent development is the appreciation that bacterial colonization of the stomach may be an important source for aspirated bacterial inocula.[120]

Gastric stress ulcers are a common complication of serious illness, but many common therapeutic approaches, such as administration of antacids or H2-antagonists, have the undesirable side effects of reducing gastric acidity and allowing bacterial proliferation. Several studies have documented that the use of sucralfate, which does not increase the pH of the stomach, results in lower rates of nosocomial pneumonia.[121,122] Other studies, however, also conducted in a prospective and double-blind fashion, failed to demonstrate any difference in pH, bacterial colonization or the development of pneumonia between ICU patients receiving sucralfate versus antacids.[123] The reader is referred to a recent review by Bonten and colleagues,[124] which probes these disparities in reports while offering several insightful explanations.

An additional group of risk factors includes conditions that reduce the effectiveness of pulmonary defense mechanisms, such as chronic obstructive lung disease and smoking. Alcohol intoxication and anesthesia may increase the risk of pneumonia by increasing the frequency of aspiration, but experimental intoxication of rabbits suggested many years ago that interference with migration of leukocytes into the air spaces may be an additional mechanism for enhanced susceptibility.[115] Although viral infections were not identified as a risk factor in these studies, bacterial superinfection has been a long recognized complication of influenza epidemics and also occurs in sporadic infection.[125,126] In fact, mortality tables compiled by federal health authorities have traditionally combined pneumonia and influenza data.[127,128] Experimental studies in mice suggest that one mechanism for adverse effects of viral infection may be impairment of lung clearance of inhaled particles, presumably related to the destruction of ciliated epithelium.[53]

In addition to factors that compromise anatomic and epithelial defenses, diseases or therapy that compromise inflammatory and immunologic defense mechanisms are of critical importance. These diseases may be hereditary deficiencies in immunoglobulins[129–131] or in phagocytic function.[132] More commonly, neoplastic disease is the cause of the immunosuppression, either directly or indirectly as a result of antitumor chemotherapy.[133,134] Patients who have received immunosuppressive therapy after organ transplantation represent another important risk group.[135–137] Although compromise in specific immunologic defenses is undoubtedly important, concomitant neutropenia is perhaps the most important risk factor.[138] The diminution in inflammation may cause atypical clinical presentations. Radiologic findings, the equivalent of gross pathologic examination, may be altered dramatically by the minimal inflammatory response.[139,140]

Human immunodeficiency virus (HIV) infection has now joined the ranks of important immunosuppressive conditions. Although attention has been focused on fungal, parasitic, and viral infections in patients with the acquired immunodeficiency syndrome, bacterial infections are also significantly increased.[141–147] The pathogens that infect patients with HIV infection are the same colonizing bacteria that cause community-acquired and nosocomial pneumonia in immunologically competent patients: *S. aureus, S. pneumoniae, H. influenzae, Moraxella* (*Branhamella*) *catarrhalis,* etc.[148] The clinical and radiographic presentations of the disease may be atypical, however, perhaps because of the prophylactic use of trimethoprim-sulfamethoxazole for prevention of *Pneumocystis jiroveci* (*carinii*) pneumonia.[142] The radiographic picture of bacterial pneumonia in patients with acquired immunodeficiency syndrome may actually mimic *Pneumocystis* infection.

TABLE 8.5. Risk factors for bacterial pneumonia

Reference	Craven[a] et al.[119]	Garibaldi[a] et al.[827]	Corensek[a] et a1.[828]	Celis[a] et al.[829]	Torres[a] et a1.[823]	England et al.[830]	George et al.[831]
Year	1986	1981	1988	1988	1990	1981	1998
Number of patients	233	520	50	118	322	—	358 (or 28?)
Setting	Intensive care with continuous mechanical ventilation	Postoperative pneumonia	Cardiac transplantation	Nonneutropenic hospitalized patients	Mechanically ventilated patients	Legionella pneumonia vs. U.S. population	Intensive care with ventilator-acquired pneumonia
Risk factors (p/odds ratio)	Intracranial pressure monitor ($p < .002$)	Low serum albumin ($p < .005$)	Posttransplant reintubation ($p = .009$)	Chronic lung disease ($p < .0003$)	More than one intubation ($p < .000012$)	End-stage renal disease (odds ratio = 340)	Admission serum albumin <2.2 g/dL (p =.0013)
	Cimetidine therapy ($p < .01$)	High anesthesia risk ($p < .0001$)	High doses of steroids ($p = .02$)	Depressed consciousness ($p < .0002$)	Prior episode of gastric aspiration ($p < .00018$)	Immunosuppression (odds ratio = 26)	Maximum PEEP ($p < .012$)
	Fall–winter hospitalization ($p < .04$)	History of smoking ($p < .001$)		Nasotracheal or orotracheal intubation ($p < .0001$)	Ventilation >3 days ($p < .015$)	Cancer (odds ratio = 11)	Absence of antibiotic therapy ($p < .0054$)
	Ventilator circuit changes q24h	Longer preoperative stays ($p < .0001$)		Large-volume aspiration ($p < .003$)	Chronic lung disease ($p < .048$)	Chronic lung disease (odds ratio = 3.7)	Colonization of upper airway by gram-negative bacilli ($p = .028$)
		Longer operative procedures ($p < .0001$)		Thoracoabdominal surgery ($p < .0018$) Age > 70 years ($p < .04$)		Smoking (odds ratio = 1.9)	Smoking history (p = .012)
		Thoracic or upper abdominal surgery ($p < .0001$)				Diabetes mellitus (odds ratio = 1.3)	Duration of mechanical ventilation >14 days (OR = 3.4)

[a]Multivariate/logistical regression analysis performed.

A special risk group for severe pneumonia is the increasingly large elderly population.[149–152] The etiologic agents and specific risk factors are not distinctive, although gram-negative organisms may be slightly more prevalent.[153] They present ever-increasing challenges in this group of patients.

The concurrence of environmental and host risk factors is important. Arnow and associates[26] described a small epidemic of *L. pneumophila* pneumonia that was associated with nebulizers that had been filled with tap water. A statistically significant association of disease with risk factors required immunocompromised patients (corticosteroid therapy) and environmental exposure (water that was contaminated with *Legionella*). Many of the variables that are risk factors for development of pneumonia are also predictive of the prognosis in patients who have lower respiratory infection Table 8.6.

Diagnosis of Bacterial Pneumonia

Difficulties in Diagnosing Pneumonia

Characterization of the etiology of bacterial pneumonia is complicated by the difficulty of making a definitive diagnosis in individual patients. Two factors complicate the laboratory diagnosis of bacterial pneumonia. First and foremost, the most common bacterial pathogens are also found in the upper airway. Thus, specimens that are collected through the upper airway can be contaminated with oral secretions. The presence of an endotracheal tube does not ensure the absence of contamination when specimens are obtained through the tube. In fact, the presence of the endotracheal tube may lead to leakage of oropharyngeal secretions around the cuff of the tube.

The second complicating phenomenon is the presence of colonizing bacteria in the bronchial tree of patients who have chronic bronchitis and the presence of inflammatory cells elicited by irritation of the trachea from indwelling endotracheal tubes or tracheostomy tubes. The diagnosis of bacterial pneumonia is outlined in consensus guidelines issued by the Infectious Disease Society of America,[154] the Canadian Infectious Disease Society,[155] and the American Thoracic Society.[156] Documentation of lower tract disease clinically or radiographically is required if specimens cannot be obtained directly from the air spaces. Many authorities use a combination of clinical, radiologic, and microbiologic findings to characterize the certitude—definite, probable, or possible—of the diagnosis.[157]

The most sensitive and specific means for an etiologic diagnosis of pneumonia is lung biopsy, but it is rarely necessary to resort to this extreme. The diagnostic approach to most pneumonias is graded, starting with the least invasive procedure. Common bacterial agents have usually been eliminated from consideration by the time the surgical stage is reached. If lung tissue is presented to the pathologist, however, it is important that a portion of the tissue be removed for bacterial culture if the surgeon has not submitted a separate biopsy to the microbiology laboratory. Any clinical considerations that might suggest special culture media, such as the possibility of legionnaires' disease, should be conveyed to the microbiologist so that special culture media can be inoculated. It is useful to freeze at –70°C uninoculated tissue from any source so that additional microbiologic evaluations may be carried out if unsuspected histologic findings redirect diagnostic considerations.

The difficulty of making a specific etiologic diagnosis of pneumonia complicates the analysis of other diagnostic methods, as well as clinical and epidemiologic investigations. There is no adequate gold standard in this area. Lung tissue is usually not available for culture. Isolation of an organism from blood or pleural fluid provides a solid diagnosis (100% specificity), so these cultures should be performed when appropriate in seriously ill patients. Reliance on culture of sterile body fluids is insufficient, however, because many pneumonias are not associated with infectious pleurisy or bacteremia. Bacterial pneumonia is accompanied by dissemination of bacteria through the bloodstream in only 20% to 30% of cases.[158]

A variety of methods has been developed to diagnose bacterial pneumonia without surgical biopsy. Two percutaneous aspiration techniques avoid passage of the diagnostic instrument through the upper airways. Needle aspiration of pulmonary lesions that have been defined by chest radiograph has been employed primarily in children but also in adults.[159–161] The procedure may be fluoroscopically guided. Direct aspiration provides a specific diagnosis, the few false positives being derived from the overlying skin. The sensitivity of the technique is more difficult to gauge, but may be enhanced by the addition of immunologic or molecular methods, particularly in patients who have received prior antibiotic therapy.[162] The most common complication is pneumothorax, which is easily treated and not usually associated with infectious pleurisy. Serious morbidity is variously estimated to be rare[159] or frequent.[161] Although this procedure is not widely performed at the present, proponents have analyzed the cost-benefit ratio more thoroughly.[163,164] Alternatively, transtracheal aspiration has been employed to obtain uncontaminated lower respiratory secretions.[165] This technique has fallen from favor in many institutions because of occasional complications. Infrequent false-positive results were reported in studies of normal volunteers, but were as high as 21% in a clinical study.[165] The false positives may result from aspiration of oral contents during the procedure or may represent colonizing tracheal flora in patients with chronic bronchitis.

The least invasive diagnostic technique is culture and Gram-stain examination of sputum obtained by

TABLE 8.6. Prognostic factors for bacterial pneumonia

Reference	Martin et al.[150]	Celis[a] et al.[829]	Torres[a] et al.[823]	Craven[a] et al.[119]	Torres[a] et al.[818]	Fine et al.[819]	Fine et al.[832]
Year	1984	1988	1990	1986	1991	1996	1990
Number of patients	136	118	322	233	92	33,148	347
Setting	Postoperative patients	Nonneutropenic hospitalized patients	Mechanically ventilated patients	Mechanically ventilated patients	Community-acquired pneumonia	Meta-analysis	Community-acquired pneumonia requiring hospitalization
Prognostic factors for mortality	Gram-negative pneumonia ($p < .001$)	"High-risk" microbe ($p < .0007$)	Ultimately or rapidly fatal disease ($p = .0018$)	Creatinine >1.5 mg/dL (odds ratio = 3.3; $p < .0002$)	Spread of pneumonia (odds ratio = 181; $p < .0001$)	Diabetes mellitus (OR = 1.3)	Age > 65 years (OR = 1.55)
	Remote organ failure ($p < .001$)	Bilateral pneumonia ($p < .008$)	Septic shock ($p = .016$)	Admitted with pneumonia (odds ratio = 4.9; $p < .0002$)	Septic shock (odds ratio = 36; $p < .0002$)	Hypothermia (OR = 5.0)	Vital sign abnormality (OR = 2.09)
	Bilateral pneumonia ($p < .001$)	Respiratory failure present ($p < .005$)	Inappropriate antibiotic treatment ($p < .02$)	No nebulized bronchodilator (odds ratio = 4.2; $p < .0004$)	Ultimately or rapidly fatal illness (odds ratio = 6.2; $p < .0185$)	Systolic hypertension (OR = 4.8)	"High risk" pulmonary etiology (OR = 2.82)
						Tachypnea (OR = 2.9)	
	Pseudomonas-predominant ($p < .01$)	Inappropriate antibiotic therapy ($p < .02$)		Duration of ventilation (odds ratio = 1.2; $p < .005$)		Bacteremia (OR = 2.8)	Bacteremia (OR = 4.1)
	Emergent operation ($p < .01$)	Age >60 years ($p < .02$)		No abdominal surgery (odds ratio = 3.2; $p < .03$)		Neoplastic disease (OR = 2.8)	Neoplastic disease (OR = 4.98)
	Bacteremia ($p < .01$)	Underlying condition ultimately or rapidly fatal ($p < .02$)		Transferred from ward of other hospital (odds ratio = 2.9; $p < .003$)		Neurologic disease (OR = 4.6)	Mental status changes (OR = 2.57)
	Postoperative peritonitis ($p < .01$)		Coma on admission (odds ratio = 2.6; $p < .009$)			Multilobar radiographic infiltrate (OR = 3.1)	
	Respiratory acquired pneumonia ($p < .05$)						

[a]Multivariate/logistical regression performed.

expectoration or by endotracheal aspiration. Careful correlation of culture and smear with clinical and radiographic data is necessary to arrive at an informed clinical diagnosis. The problems with sputum as a diagnostic specimen have been emphasized,[166–168] but other reports have been more optimistic.[169–171] In one prospective study of sputum, bronchial aspiration, and transtracheal aspiration, the authors recovered the predominant organism from all three types of specimen.[172] Homogenization of sputum and inoculation of mice did not improve the diagnostic yield. It has been suggested that quantitative cultures of tracheal aspirates may provide better data (see the discussion of bronchoalveolar lavage, below).[173] The quantitative technique involved performing a dilution of sputum that had been liquefied enzymatically before plating onto agar. A simpler semiquantitative approach, in which the sputum was washed twice with saline and struck onto agar as usual, correlated closely with the quantitative technique. This technique has not been widely adopted, but most laboratories use the semiquantitative technique (although few go through the washing steps).

In an attempt to select specimens that were enriched for lower respiratory secretions and relatively devoid of contaminating upper respiratory flora, Murray and Washington[174] studied several screening criteria using the Gram stain. The criteria most commonly employed in clinical microbiology laboratories at present are the presence of fewer than 10 squamous epithelial cells and more than 25 polymorphonuclear neutrophils per 100 × microscopic field. The presence of macrophages documents the presence of alveolar contents, but does not increase the diagnostic usefulness of the screening criteria.[175,176] A careful comparison of expectorated sputum and transtracheal aspiration documented the usefulness of the criteria.[176,177] If more than 25 squamous epithelial cells were present in the expectorated sputum, there was agreement of bacterial isolates with transtracheal analysis in only 27% of cases. When fewer than 10 squamous cells and more than 25 neutrophils were present, a potential pathogen growing in the expectorated sputum was 92% predictive of growth in the transtracheal aspirate. When multiple potential pathogens are present in the sputum, careful correlation of the results with clinical data is necessary before the bacteria can be accepted as etiologic agents.

Assessment of bacteria in areas of the smear that are inflammatory and do not contain squamous epithelial cells provides useful information with which to interpret the results of culture. Assessment of pneumococci is particularly difficult because of the frequent presence of viridans streptococci in the specimens. Rein and colleagues[178] have assessed the effect of varying criteria on the sensitivity and specificity of the microscopic examination. The best balance of sensitivity and specificity occurred when at least 10 lancet-shaped diplococci per oil immersion field were present. While pneumococci may be confused with other streptococci, gram-negative bacteria, particularly *H. influenzae,* may be overlooked in the pink proteinaceous background. The myth that Gram-stain examination is a simple procedure is belied by studies such as the evaluation of sputum smears prepared by house staff.[179] Smears prepared by house staff were judged inadequate in 15% of cases compared to 3% for smears prepared by microbiology technologists. The sensitivity of interpretation of pneumococci by house staff was approximately 90% as judged by technologist interpretations or by culture, but there was a 50% false-positive rate. In contrast, the house staff underdiagnosed *Haemophilus* sp. on the smears.

More refined bronchoscopic techniques have been developed and applied to diagnosis. Protected specimen brushes of distal bronchioles provide the most accurate specimens.[180,181] Chastre and colleagues[182] compared the results of this technique with lung biopsy in patients immediately after death (a study made possible by a change in French law regarding postmortem examination). They found an excellent correlation between the techniques when more than 10^3 colony-forming units of a bacterium per milliliter were present. Since then, a number of remarkably similar comparison studies have been conducted on recently deceased patients. Some of these investigations demonstrated good correlation among postmortem histology, lung tissue culture results, and different bronchoscopic-guided sampling techniques.[183–185] On the other hand, many studies have failed to affirm any significant differences in diagnostic accuracy between initial clinical/radiographic findings, noninvasive specimen collection techniques, and bronchoscopic-guided sampling procedures.[186–189] It is important to remember that the quantitative threshold for positivity (a likely etiologic role for the bacterium) varies considerably between studies as well as between sampling techniques. Direct comparisons, therefore, should be made with caution. Similarly, although histologic diagnosis was considered the gold standard in all of these comparative studies, Corley et al.[190] demonstrated considerable interobserver variability among four pathologists in defining the mere presence or absence of pneumonia. Finding intracellular bacteria in more than 25% of cells[191] or demonstrating more than 10 respiratory epithelial cells[192] in fluid obtained by cytospin analysis of bronchoalveolar lavage (BAL) fluid have been proposed as indicators of pneumonia. Quantitative cultures from peripheral BAL have also been used widely.[193]

In addition, transbronchial biopsies are sometimes performed and the surgical pathologist may be asked to provide a morphologic assessment. The pattern of the inflammatory reaction may vary, depending on the timing of the biopsy, as discussed earlier. Acute lung injury in a bronchoscopic biopsy (characterized by fibrin in alveoli, interstitial edema, hyaline membranes, and

neutrophilic exudates) must always be considered infection until proven otherwise. At a minimum the surgical pathologist is obligated to perform acid-fast and fungal stains and to examine hematoxylin and eosin (H&E) sections for viral inclusions in this scenario, even when infection is not a strong clinical consideration; optimally, a Gram stain modified for tissue should be included as well. Other stains for microbes may be useful, as discussed below, but may be difficult for smaller laboratories to perform. An important diagnostic key is the presence of necrosis, even of minimal extent, because the probability of achieving a positive histochemical or immunohistochemical result increases substantially when this finding is present.[194]

The etiologic diagnosis of bacterial pneumonia, therefore, is difficult, but an informed analysis can be made when all possible means are employed. Isolation of pathogens that are not part of the normal respiratory flora is diagnostic. Unfortunately, with the exception of *L. pneumophila* and *Mycobacterium* sp., such instances are extremely rare.

Morphologic Detection and Identification of Bacteria

The Gram stain provides important information for the assessment of bacterial processes. Modifications of this procedure for histologic sections, most prominently the Brown and Brenn and Brown–Hopps procedures, have been developed, but these stains for histologic sections are much more difficult to evaluate than are Gram-stained smears. The cut surface of lung tissue may be touched lightly to the surface of a flamed glass slide so that the sterility of the tissue is maintained. Alternatively, a sterile scalpel blade may be drawn across the surface of the lung to collect exudate, which is then spread on a glass slide. The scrape technique is particularly effective when the exudate is fibrinous. Extra slides should be prepared to allow for a subsequent need for special examinations, such as calcofluor white staining for fungi, acid-fast stains, or immunofluorescence examination for selected pathogens. To wait for sections of paraffin-embedded tissue before doing special stains for microbial pathogens is analogous to waiting to examine postmortem lung tissue with the electron microscope when an open lung biopsy could have been fixed promptly in glutaraldehyde solution.

If the tissue has already been fixed in formaldehyde solution, several histologic stains can be used to demonstrate and characterize bacterial pathogens in tissue sections. The most useful stains and their diagnostic applications are listed in Table 8.7

Bacteria have been characterized traditionally by their morphology (round = coccus/cocci; elongated = bacillus/bacilli; intermediate = coccobacillus/coccobacilli) and by their resistance to decolorization in Gram stain (gram-positive = blue, resists decolorization; gram-negative = red, decolorizes readily).[37] A visual guide to bacterial morphology in Gram stain and the associated differential diagnosis are presented in Figure 8.9.

Modified Gram stains such as the Brown and Brenn, Brown–Hopps, and MacCallum–Goodpasture procedures are used to differentiate gram-positive and gram-negative bacteria in tissue, including the gram-positive agents of actinomycosis and nocardiosis. In our experience, the Brown–Hopps stain is superior for demonstrating gram-negative bacteria and is the best single choice for a tissue Gram stain. On the other hand, the Brown and Brenn procedure colors gram-positive bacteria somewhat better than the other methods.

Grocott's methenamine silver procedure, which is usually employed for the demonstration of fungi, also stains *Actinomyces* and related organisms, *Nocardia* spp.,

TABLE 8.7. Special stains for bacteria in clinical specimens

Indication	Primary stain	Alternatives	Comments
General bacteria	Brown–Hopps	Brown and Brenn	Gram's stain of imprint preferred
Gram-positive bacteria	Brown and Brenn	Brown–Hopps	Gram's stain of imprint preferred
Gram-negative bacteria	Brown–Hopps	Brown and Brenn	Gram's stain of imprint preferred
Poorly staining bacteria	Steiner[a]	Warthin–Starry, Dieterle[a]	Distorts morphology; no gram reactivity
Acid-fast bacilli	Ziehl–Neelsen	Auramine, auramine-rhodamine	Should be used as general screen; Ziehl–Neelsen or Kinyoun on imprint preferred
Partially acid-fast organisms suspected	Fite–Farraco	Putt	Modified Kinyoun on imprint preferred
Actinomyces	Brown and Brenn	Gomori's methenamine silver	
Nocardia	Brown and Brenn; Fite	Grocott's methenamine silver	
Legionella, Yersinia, Francisella, Brucella	Steiner, Warthin–Starry, or Dieterle	Brown–Hopps	Gram's stain on imprint useful

[a]The silver impregnation stains are essentially interchangeable. The preferred stain is the one for which the output of the local laboratory is most satisfactory.

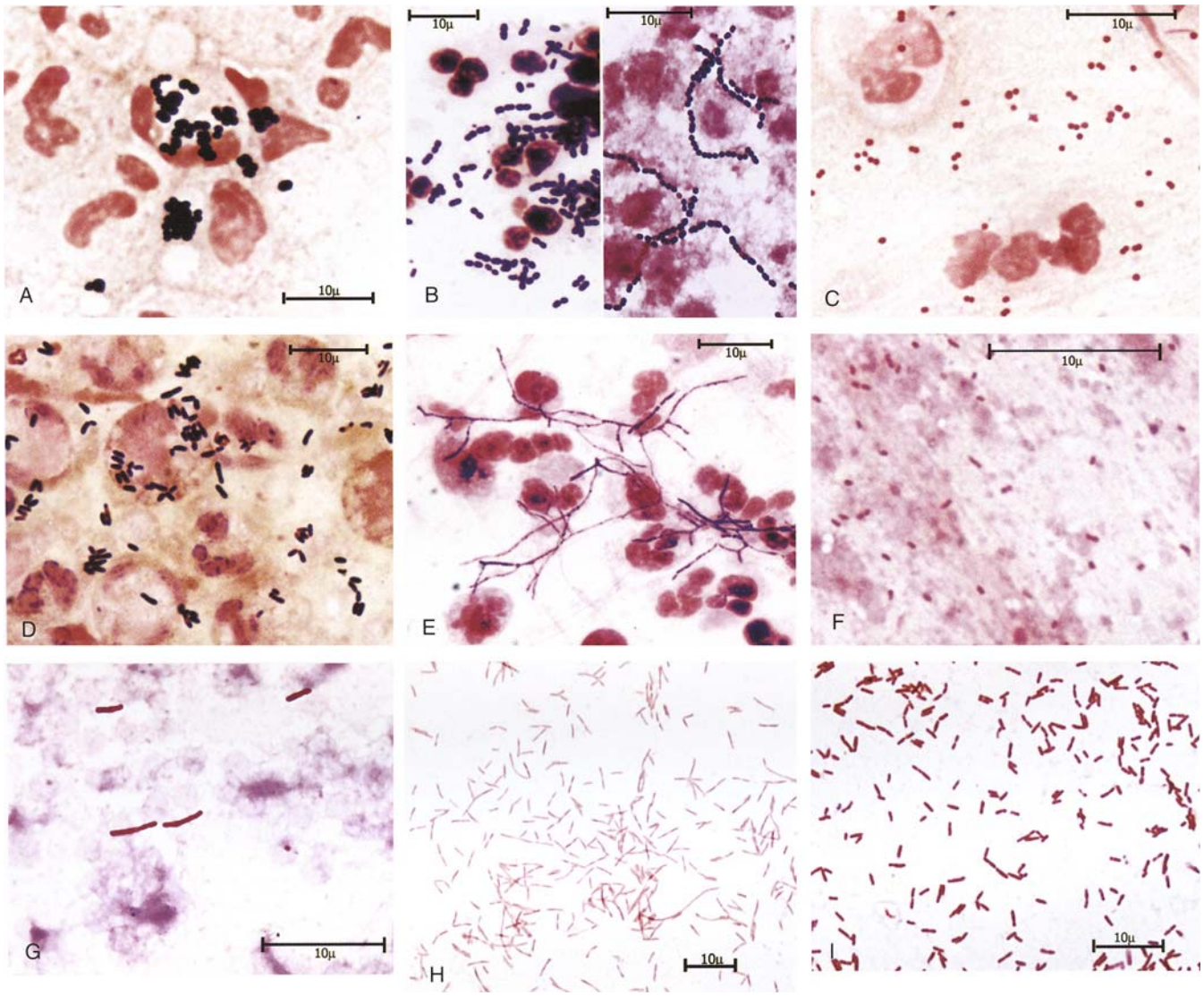


FIGURE 8.9. **A.** Gram-positive cocci in clusters: *Staphylococcus aureus.* **B.** Gram-positive cocci in pairs/chains. Left: *Streptococcus pneumoniae.* Right: *Streptococcus pyogenes.* **C.** Gram-negative diplococci. *Neisseria meningitidis, Neisseria gonorrhoeae, Moraxella catarrhalis.* (Technically, *Moraxella catarrhalis* has been placed in a bacillary genus, although this organism does have coccal morphology and responds as a coccus in the penicillin test.) **D.** Short gram-positive bacilli/coccobacilli: *Corynebacterium jeikeium, Listeria monocytogenes.* **E.** Filamentous gram-positive bacilli: *Nocardia* spp., *Actinomyces* spp., *Rhodococcus equi, Bartonella henselae* (sometimes filamentous). **F.** Gram-negative coccobacilli: *Haemophilus influenzae, Acinetobacter baumanii.* **G.** Large gram-negative bacilli: *Klebsiella pneumoniae, Escherichia coli, Serratia marcescens, Salmonella typhi, Yersinia pestis, Proteus mirabilis, Proteus vulgaris, Enterobacter* spp., *Salmonella* spp., *Yersinia enterocolitica.* **H.** Faintly staining gram-negative bacilli: *Legionella* spp., *Francisella tularensis, Brucella* spp., *Bordetella* spp. **I.** Long slender gram-negative bacilli: *Pseudomonas aeruginosa, Burkholderia pseudomallei, Burkholderia cepacia.* (Bacterial gram stain montage courtesy of Dr. A. E. McCullough, S. Stewart, and L. Burdeaux, Mayo Clinic Hospital Microbiology Laboratory, Scottsdale, AZ.)

and nonfilamentous bacteria that have polysaccharide capsules such as *S. pneumoniae, K. pneumoniae, H. influenzae, Rhodococcus equi,* and certain strains of *Neisseria meningitidis.* If the incubation period is prolonged, mycobacteria, including *M. tuberculosis,* may be stained by the Grocott method.

Silver impregnation stains such as the Steiner, Dieterle, and Warthin–Starry procedures are required to demonstrate *Calymmatobacterium granulomatis* and spirochetes such as *Treponema pallidum, Borrelia burgdorferi,* and *Leptospira* spp. The silver impregnation procedures stain all bacteria nonselectively and are excellent for demonstrating small, weakly gram-negative bacilli, such as *Legionella* spp., *Francisella tularensis* (tularemia), *Afipia felis* (proposed as an etiologic agent of cat-scratch disease), *Pseudomonas pseudomallei* (melioidosis), and *Bartonella*

(formerly *Rochalimaea*) *henselae* (cat-scratch disease and bacillary angiomatosis).[195] The accretion of reduced silver on these bacteria greatly increases their visibility but also enlarges and distorts the form of the bacteria. When compared to the Gram stain, silver impregnation procedures provide much greater sensitivity for detecting small numbers of either gram-positive or gram-negative bacteria. Once bacteria are detected, a modification of the Gram stain can be applied to replicate tissue sections to determine Gram reactivity of the organisms.

Most mycobacteria, particularly the *Mycobacterium avium* complex, not only appear acid fast after staining with the Ziehl-Neelsen procedure, but also are weakly gram positive and stain positively with the periodic acid–Schiff (PAS)[196] and Grocott's methenamine silver procedures in fixed paraffin-embedded tissue sections. (See also Chapter 9.) Auramine or auramine-rhodamine may be used to screen sections efficiently for acid-fast bacilli if a fluorescence microscope is available. A mercury light source is not required, as halogen lamps emit light efficiently in the exciting range for these dyes. The fluorescent methods are more sensitive than the Ziehl–Neelsen procedure and it is easier to screen sections quickly, but it is more difficult to assess the morphology of putative bacilli and to characterize correctly nonbacterial acid-fast material that may be abundant in sections. The routine acid-fast stain for most laboratories should be the Ziehl–Neelsen procedure, unless an observer is familiar with the fluorescent stains. A modified decolorization procedure should be employed only if a partially acid-fast bacterium is suspected. It should be noted that the fluorescent acid-fast stains operate on the same principles as the Ziehl–Neelsen stain; they are not immunologically specific procedures.

Nocardia spp., *Legionella micdadei,* and *R. equi* are weakly acid fast and nonalcohol fast. In addition, the mycobacterial rapid growers such as *Mycobacterium fortuitum* and *Mycobacterium chelonae* may not be stained by the Ziehl–Neelsen procedure. Therefore, modified acid-fast procedures that use an aqueous solution of a weak acid, such as 1% sulfuric acid, as a decolorizer, are required to stain these bacteria satisfactorily. The decolorization provided by the Putt modification of the acid-fast stain is more gentle than that provided by the Fite or Fite–Farraco procedures. Instances have been reported in which the Putt modification produced acid-fastness in *Actinomyces* spp., normally not considered acid-fast bacilli, whereas the Fite stain produced negative results.[197] For this reason the Fite modification is the preferred procedure. Rarely, *L. pneumophila* may appear acid fast in tissue (personal observation). It is important to recognize that *Legionella* spp. are acid fast only in tissue and do not appear acid fast after isolation on agar media.

When immunologic reagents of adequate sensitivity and specificity are available, either immunofluorescence or immunoenzymatic staining of bacterial pathogens in smears or formalin-fixed deparaffinized tissue sections can be routinely used to extend the diagnostic capability of conventional histopathology. Immunohistologic staining is invaluable for confirming a presumptive diagnosis, especially when only fixed tissues are available, or for identifying a bacterium in a contaminated specimen. The limited commercial availability of many of the reagents limits the usefulness of this potentially important diagnostic approach.

Controls for histologic stains should be carefully selected to test the reliability of the reagents and the stain procedure. A section that is teeming with acid-fast bacilli is a very poor guarantor of adequate staining in a marginally positive specimen. On the other hand, it is not practical to include a positive control in which scant bacteria are present, because much time will be wasted in searching and there is a finite probability that bacteria will not be visualized even though the stain worked well. Therefore, a middle ground must be sought in which a moderate but not overwhelming number of organisms is present. It is not a good idea to use an "all-purpose" control, such as colonic material that contains mixed morphotypes. An appropriately screened control that contains gram-positive cocci and gram-negative bacilli may be useful, however. Jung[198] described a method for creating fibrin substrates into which cultured bacteria may be placed before fixation and embedding. If the stain is intended to detect a "difficult" organism, such as the Brown–Hopps stain for *Legionella,* it is important to use a control that matches the task; good coloration of *E. coli* is no assurance that *Legionella* will be visualized similarly.

Macroscopic Distribution, Inflammatory Characteristics, and Time Course

Macroscopic Distribution

The classic pathologic characterization of pneumonia has been by macroscopic distribution. At the two poles are lobar and bronchopneumonia.

Lobar Pneumonia

Lobar pneumonia was described as the manifestation of pneumococcal pneumonia. As the name implies, the inflammatory process consumes the greater part of a lobe of the lung and may involve the entire lobe. Typically, the consolidative process extends to and is sharply delimited by the pleura (Fig. 8.10) or by a major fissure (Fig. 8.11). Macroscopic consolidation occasionally may breach the boundary of the lobe. Osler considered the involvement of a single lobe to be an important diagnostic criterion of

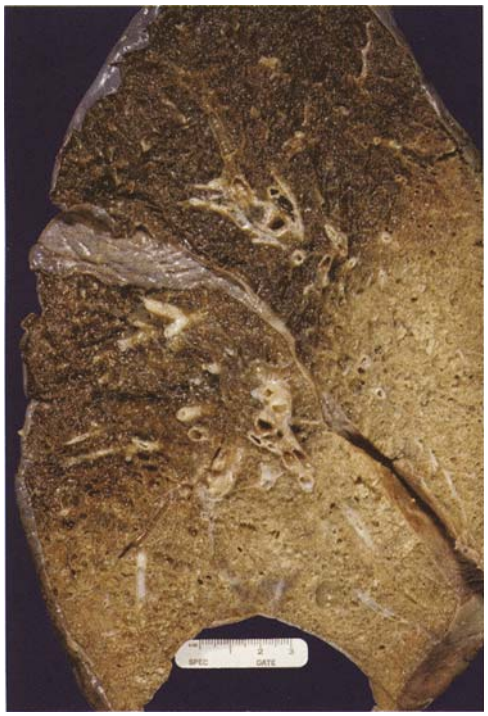


FIGURE 8.10. Lobar pneumonia. Elderly man found by his family in unresponsive state died shortly after arriving at the emergency department. *Streptococcus pneumoniae* was isolated in pure culture from the postmortem lung; extensively emphysematous air spaces are full of very cellular exudate (stage of gray hepatization). Exudate does not involve entire lobe but extends by contiguity to both pleural surface and lobar fissure. This case is unusual in that a major portion of two lobes is involved, but the entire process is contiguous and additional foci are not present in other areas of the lung.

lobar pneumonia.[1] Microscopic inflammation often extends across the fissure and into the pleura, producing a roughened pleural surface, full-blown pleurisy, or even empyema (Fig. 8.12).

Although lobar pneumonia is classically associated with *S. pneumoniae,* other pathogens may produce a similar pathologic picture. A typical lobar inflammation caused by *K. pneumoniae,* type 1, is sometimes referred to as Friedländer's pneumonia (Fig. 8.11). A minority of cases of *L. pneumophila* infection may also produce a typical lobar configuration. Individual cases of lobar pneumonia have been ascribed to *S. aureus,*[199] *Neisseria gonorrhoeae,*[200] and *M. pneumoniae.*[201]

The pathologic process of lobar pneumonia (primarily pneumococcal) was delineated as early as the first decades of the 19th century by Laennec, who described the basic progression of the consolidative process.[202] The inflammatory process proceeds through stages of edema, red hepatization, gray hepatization, and resolution.[203] The frequency of various stages and manifestations of lobar pneumonia were tabulated in a series of 400 cases by Berry[204] (Table 8.8). It is noteworthy that two or more stages in the process of consolidation may coexist in the same lung (Fig. 8.11). The pneumonic process is dynamic, so that the inflammatory process is more advanced in the areas infected first than in recently infected areas.

Heffron[202] described the sequence of inflammatory events in lobar pneumonia as follows:

Engorgement

It is rare to find lungs that are totally in the stage of engorgement at autopsy. Rather one must examine the edges of older advancing lesions. The lung is heavy and doughy, but still crepitant. A frothy blood-tinged fluid exudes from the cut surface. Microscopically, the capillaries are engorged, the alveolar epithelium is swollen, and the air spaces contain edema fluid, red blood cells, and

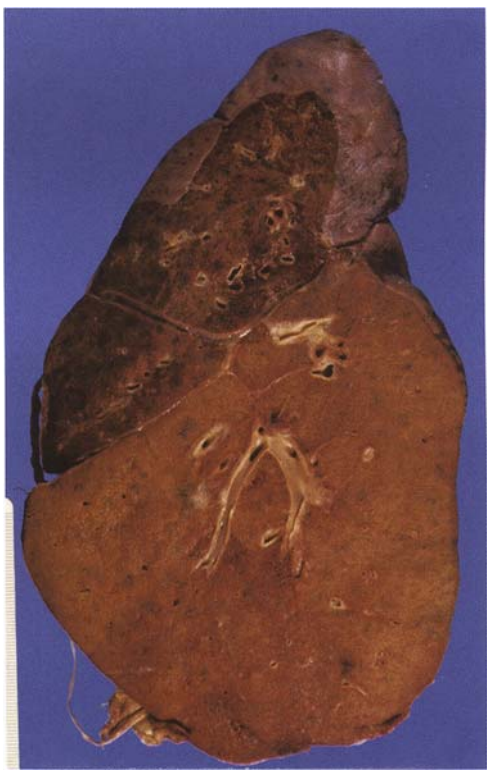


FIGURE 8.11. Friedländer's lobar pneumonia. An elderly alcoholic was admitted to the hospital with acute pneumonia that progressed rapidly. *Klebsiella pneumoniae* type 1 was isolated in pure culture from antemortem sputum and postmortem lung tissue. The entire right lower lobe is consolidated; inflammation extends both to pleural surfaces and the lobar fissure. The volume of the lung is expanded; some areas of the lobe are beefy red while others are yellow-white (stages of red and gray hepatization).

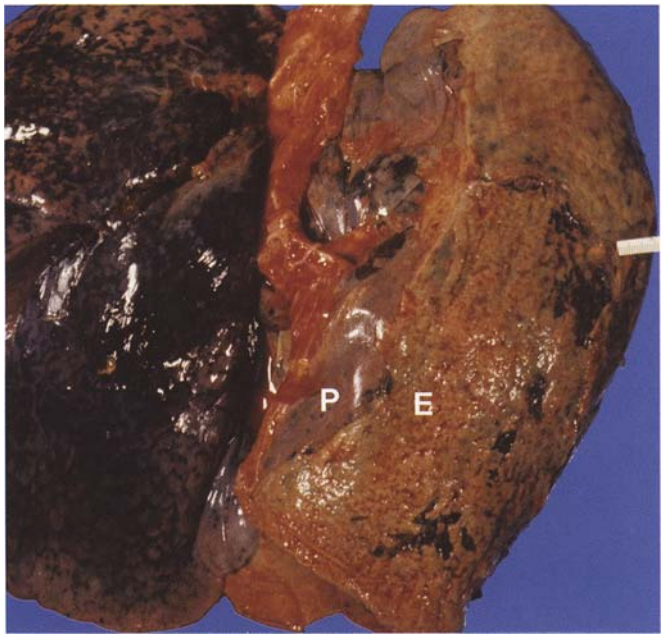


FIGURE 8.12. Anaerobic empyema. The right lung is encased in a thick greenish exudate E., which originated in an underlying necrotizing pneumonia. Portions of the glistening pleura (P) remain visible.

desquamated epithelium. This stage probably lasts only a few hours in the usual case.

Red Hepatization

The lung is heavy, noncrepitant, and no longer floats on water. The surface is dark red or reddish-brown in color. In consistency it is dry and granular because of fibrin deposition in the air spaces. When the etiologic agent is heavily encapsulated, such as *S. pneumoniae* type 3 or *K. pneumoniae* type 1 may be, the exudate has been described as more viscid than usual. Microscopically, the air spaces are filled with a fibrin mesh containing erythrocytes, polymorphonuclear and mononuclear leukocytes, and desquamated epithelial cells. The cellular exudate is remarkably intact and well preserved.

TABLE 8.8. Pathologic features of lobar pneumonia in 400 autopsy cases

Pathologic state	Number of cases
Red hepatization	212
Red and gray hepatization	225
Gray hepatization	317
Organizing pneumonia	2
Combination of above	44
Abscess or gangrene	19
Infarct	6

Source: Berry.[204]

Gray Hepatization

At this stage the lung is dense and friable, gray to white to yellow in color. The cut surface is somewhat more moist than in the previous stage and exuded fluid is turbid. Microscopically, the predominant cell in the alveolar exudate is the polymorphonuclear neutrophil. Most erythrocytes have lysed and the fibrin net is less obvious. At this stage the inflammatory exudate is undergoing lysis. Cells stain poorly, their outlines are indistinct, and hemosiderin pigment may be evident. The interstitial blood vessels are no longer engorged and occasionally megakaryocytes, released from the bone marrow during the outpouring of leukocytes, may be trapped in the capillaries. In pneumococcal lobar pneumonia the bacteria stain well and are still viable during the stage of red hepatization, but are poorly stained and nonviable once the gray hepatization phase is reached.

The time course for the inflammatory process is variable, with the duration of the red and gray hepatization phases being estimated at 2 to 3 days each. The time of maximum consolidation has been variously estimated to be 2 days[205] or 3 to 6 days.[206] The physical effects of the extensive inflammatory consolidation are emphasized by the observation that an artificial pneumothorax produces little, if any, collapse of the affected lung.[207]

Focal Pneumonia

The other major anatomic pattern is best described as focal pneumonia, but has also been called lobular pneumonia, bronchopneumonia, bronchial pneumonia, or bronchiolar pneumonia. The latter names illustrate the association of the inflammation with the bronchial tree, but they are misnomers because the inflammatory process is essentially a disease of the air spaces—the respiratory bronchioles, alveolar ducts, and alveoli. Lobular pneumonia is an accurate description of the most common variant of focal pneumonia. A paper-mounted whole-lung section (Gough section) illustrates clearly the association of inflammation with the centrilobular portion of the pulmonary lobule (see Fig. 8.3). The heavy carbon deposition in the patient, who was a cigarette smoker, clearly delineates the terminal bronchioles at the center of the lesions. The pathogenetic mechanisms are similar to those at work in lobar pneumonia. The stages of consolidation are not so well described as in lobar pneumonia, but undoubtedly follow a similar pattern. Spread of the infection is probably through the bronchial tree from lobule to lobule, segment to segment, and lobe to lobe. Extension from alveolus to alveolus through the pores of Kohn probably occurs also.

The differentiation of lobar from focal lobular pneumonia is not always clear and the processes probably represent a continuum rather than dichotomous entities. When focal pneumonia is extensive, differentiation from lobar pneumonia may be difficult or impossible,[208] a fact

that has been recognized for many decades.[202] Such lesions are described as confluent lobular pneumonia (see Fig. 8.3). Consolidation may appear lobar by radiographic analysis[209] but be characterized as confluent lobular pneumonia when examined at autopsy.[210]

Similarly, the etiologic associations are not precise. *S. pneumoniae* is the classic agent of lobar pneumonia, but frequently produces focal consolidation. Conversely, other agents may on occasion cause a lobar consolidation. Although *S. aureus* pneumonia is usually described as a focal process, Chartrand and McCracken[199] described a radiographic picture of lobar pneumonia as the most frequent presenting manifestation of infection in 79 infants and children. A factor in characterizing the infections may be the relative imprecision of chest radiographs in defining the exact anatomic distribution of infiltrates. The radiograph is the only tool that clinicians have to assess the distribution in most cases, however, so it is important to realize the limitations of the technique.

Round Pneumonia

A variant of focal pneumonia has been given the descriptive designation "round pneumonia" (Fig. 8.13). This distribution may be caused by a variety of infectious agents and is not distinctive in a pathogenetic sense. The lesions presumably result from centrifugal spread of the inflammatory process. They may become quite large (Fig. 8.14) and may be multiple (Fig. 8.15). Round pneumonia has been described primarily in children, but may also occur in adults.[211,212]

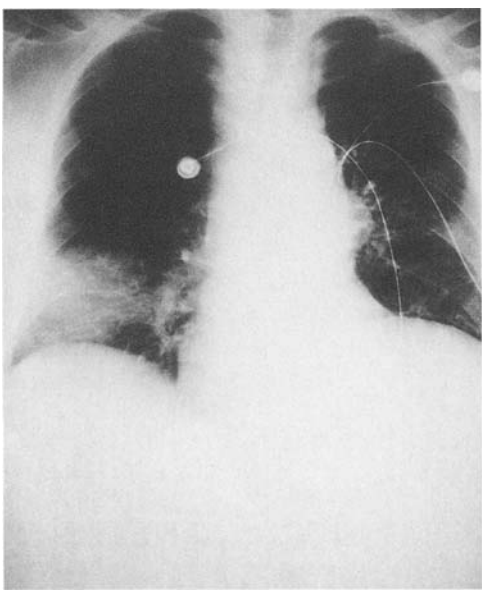


FIGURE 8.13. Round pneumonia. Chest radiograph of patient with *Haemophilus influenzae* pneumonia illustrates single, round, demarcated focus of consolidation in lower lobe. Various infectious agents may produce this appearance.

FIGURE 8.14. Round pneumonia. Very large, poorly demarcated consolidated nodule. *Legionella pneumophila* serogroup 1 was isolated in pure culture from postmortem lung. Nodule extends to pleural surface; second pleural-based lobular focus is seen in the lower part of the lobe (arrow). Such pleural-based lesions may suggest the possibility of a pulmonary embolus to the radiologist early in the course.

FIGURE 8.15. Round pneumonia. Multiple poorly marginated pulmonary nodules are present in this lung section. *Legionella pneumophila* serogroup 4 was isolated in a pure culture from the lung at autopsy. The cut surface of the lung had a very dry, granular appearance caused by a combination of fibrin and lysed inflammatory cells in the air spaces. Differential diagnosis includes fungal pneumonia, and radiographically neoplastic nodules must be considered.

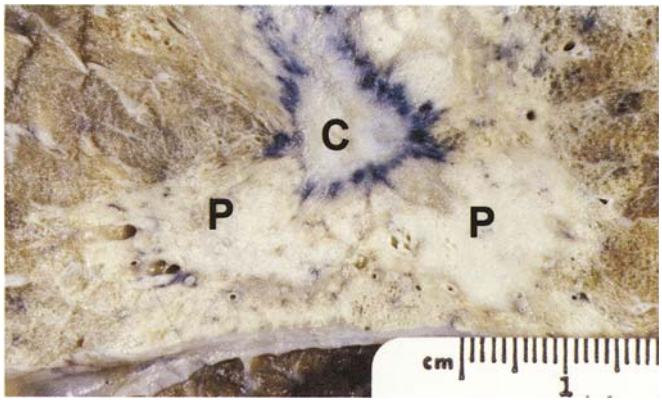


FIGURE 8.16. A poorly demarcated nodule of pneumonia (P) is intermixed with pulmonary metastasis from carcinoma of the bladder C. *Legionella pneumophila* serogroup 1 was isolated in a pure culture from the postmortem lung tissue.

The radiographic differential diagnosis of round pneumonia is radiation pneumonitis, round atelectasis, inflammatory pseudotumor, and metastatic neoplastic lesions.[212] If the inflammation reaches the pleura, the early phases of a septic pulmonary infarct must be considered. When examined macroscopically, the lesions can usually be differentiated from neoplastic nodules, but fungal pneumonia must be considered in the differential diagnosis (Fig. 8.16).

Necrotizing Pneumonia and Lung Abscess

Proteolytic and elastolytic enzymes released by bacteria or inflammatory cells may produce physical destruction of lung parenchyma as part of the inflammatory process. The classic studies of pneumococcal pneumonia emphasized resolution of infection without complications, but later investigations have emphasized the presence of necrotizing infection and abscess formation even with this pathogen.[213] Most commonly, necrotizing pneumonia is caused by bacteria that produce abscesses in other situations, such as *S. aureus,*[214,215] *S. pyogenes,* enteric gram-negative bacilli,[216,217] and *P. aeruginosa.*[218]

The necrotizing nature of the process may only be recognized on pathologic examination. Approximately 20% of *L. pneumophila* pneumonias have macroscopically demonstrable abscesses,[211] but they are rarely large enough to be visible in chest radiographs.[219,220] Virtually any bacterium may produce necrotizing pneumonia with the formation of abscesses on occasion. Viridans (α-hemolytic) streptococci are not usually considered primary pathogens, but one group of these organisms has been firmly established as a unimicrobial cause of abscesses in multiple organs, including the lung.[221,222] These organisms are variously referred to by investigators in Britain and the United States as *Streptococcus milleri* or *Streptococcus anginosus.* One group of investigators has described the presence of elastin fibers in KOH preparations of sputum as a more sensitive documentation of necrotizing pneumonia than radiographic visualization of cavities.[223]

Necrotizing pneumonia and lung abscess in nonhospitalized patients are often associated with mixed infections of anaerobic bacteria and aerobic members of the oral microflora.[35,36,224] The lesions are often large and single (Fig. 8.17). They are associated with episodes of substantial aspiration and are thus located in dependent portions of the lung, such as the superior segment of the lower lobe and the apical segment of the upper lobe. Virtually any species of anaerobe may be present. Typically, *Fusobacterium nucleatum, Prevotella* spp. or *Porphyromonas* sp. (formerly *Bacteroides melaninogenicus* group), and *Peptostreptococcus* sp. are isolated. Although *Bacteroides fragilis* is not usually isolated from the upper airways, this important pathogen is present in a minority of lung abscesses (see also Chapter 5).

Inflammatory Characteristics and Time Course

Acute Pneumonia

Most bacterial pneumonias, excepting mycobacterial infection, are an acute inflammatory process in

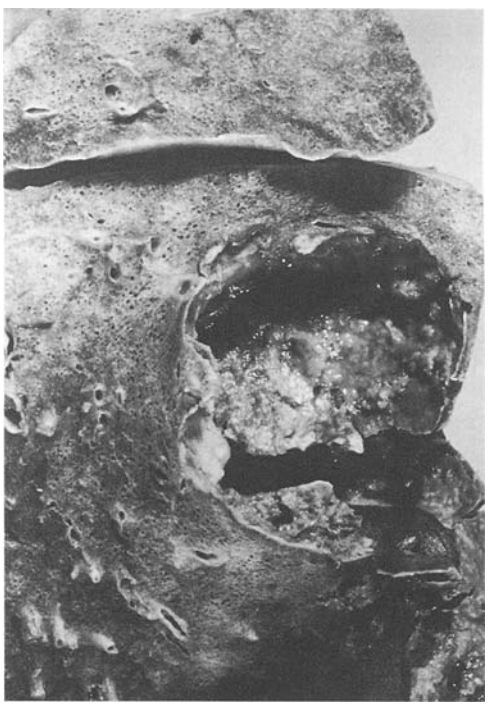


FIGURE 8.17. A lung abscess caused by mixed aerobic and anaerobic bacteria after aspiration of oropharyngeal contents. Single thick-walled cavity is located in the superior segment of the lower lobe.

which the polymorphonuclear neutrophil plays a prominent role. The preceding description of classic lobar pneumonia represents such a process. The development of the inflammatory process requires from 5 to 10 days, and acute symptoms persist a similar period in 70% of untreated cases.[202] Rarely, symptoms last only 2 to 3 days, and occasionally they persist for 2 to 3 weeks.

The characteristics of the inflammatory process are somewhat more variable. Cases of well-documented legionnaires' disease have been described in which the histologic reaction in biopsied lung resembled acute diffuse alveolar damage with prominent hyaline membranes and without a prominent accumulation of polymorphonuclear neutrophils in the air spaces.[225] Approximately one third of *L. pneumophila* infections are characterized by an alveolar exudate exclusively of macrophages.[211] Walsh and Kelley[226] described an unusual case of legionnaires' disease in which the exudate was exclusively mature plasma cells. The clinical presentations and time course in these cases with atypical inflammatory cell populations did not differ from a typical case.

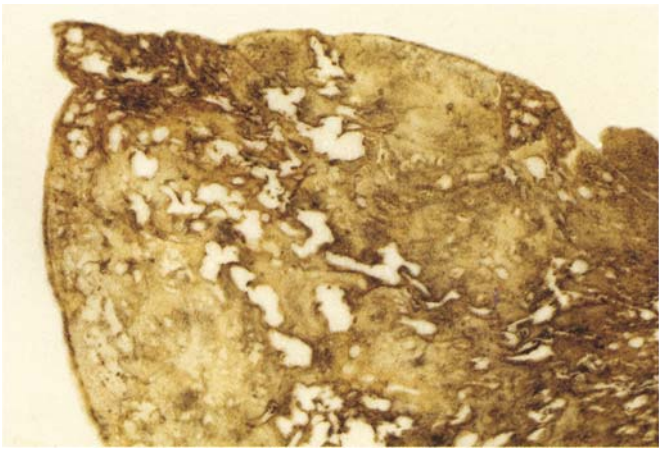


FIGURE 8.18. An extensive area of fibrosis is evident in a paper-mounted whole-lung section (Gough technique) of a patient who had had legionnaires' disease. Distal bronchioles are prominently outlined by fibrosis. *Legionella pneumophila* was isolated antemortem.

Chronic Pneumonia

Chronic pneumonias are most commonly caused by fungi and mycobacteria, but some bacterial species frequently produce a chronic process in which islands of acute inflammatory cells are mixed with chronic inflammation and fibrosis. *Actinomyces* spp. and related bacteria, *Nocardia* spp., and *Pseudomonas pseudomallei* typically produce such infections. It should be emphasized, however, that other pathogens typically associated with acute inflammation may on occasion produce a chronic process.

Organizing Pneumonia and Fibrosis

Most episodes of acute pneumonia resolve spontaneously or after antimicrobial chemotherapy by resorption of the inflammatory exudate with restitution of the normal underlying structure. In some cases, however, the stimulation of fibroblasts by the inflammatory response elicits a proliferation of connective tissue (Fig. 8.18). Kuhn[227] defined at least four pathologic processes that contribute to the remodeling of the lung: interstitial thickening, deposition of a connective tissue matrix within the air spaces, collapse of the air spaces, and contraction of the wound. The intraalveolar proliferation of fibroblasts produces nodular structures called Masson bodies (Fig. 8.19). (Also see Chapter 4 for the idiopathic entity BOOP, now preferably referred to as cryptogenic organizing pneumonia.) Pulmonary myofibroblasts participate in the contractile phase of pulmonary fibrosis,[228–230] producing a contracted residuum of mature fibroblasts.[231] The process of pulmonary fibrosis is discussed in detail in Chapter 19.

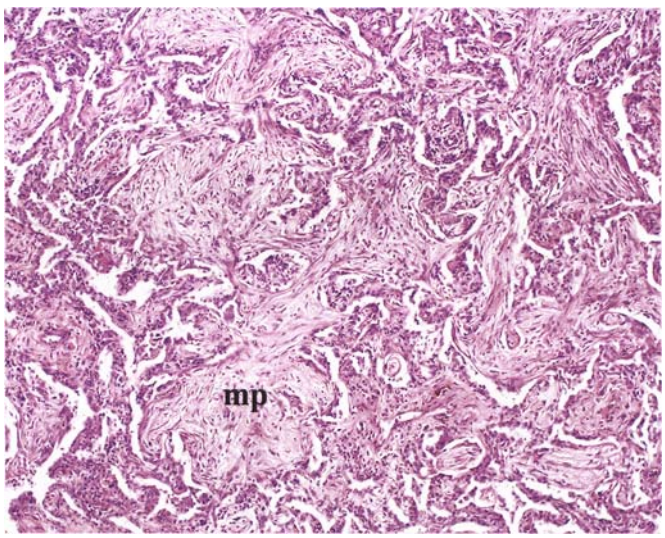


FIGURE 8.19. Organizing pneumonia. The dominant microscopic feature of organizing pneumonia is the so-called Masson polyp (mp). Similar to the myofibroblastic proliferation of the proliferative phase of diffuse alveolar damage (Fig. 8.8), organizing pneumonia is characterized by proliferation of myofibroblasts within the alveolar spaces. Reactive type 2 cells can be seen typically covering the surface of these polyps. Some organizing infectious pneumonias heal without a residual scar or structural remodeling (e.g., pneumococcal pneumonia), while others resolve with extensive scar formation.

Specific Etiologic Agents of Pneumonia

Gram-Positive Cocci

S. pneumoniae

The pneumococcus is the king of pulmonary pathogens, although its relative position has been diminished by addition of new pathogens and new risk factors. The clinical,[232–234] epidemiologic,[235] pathogenetic,[58,73,203,236] diagnostic,[237] and pathologic[202] aspects of pneumococcal pneumonia have been reviewed and discussed extensively. A variety of pneumococcal serotypes produce infection, but a select group of serotypes, which have been incorporated into pneumococcal vaccines, produces the majority of infections.[238] The recent development of pneumococcal strains resistant to multiple antibiotics, including penicillin, introduces a new therapeutic concern into the treatment of pneumococcal pneumonia.[239,240] While the proportion of resistant strains remains low in the United States compared to other countries,[241] the number of invasive infections caused by penicillin nonsusceptible strains is on the rise.[242] The seriousness of the problem is increased by the frequent association of resistance to other antimicrobial agents, such as trimethoprim-sulfamethoxazole, cefotaxime, erythromycin, and tetracycline.[240] On the other hand, preliminary evidence suggests that the clinical outcome of infections caused by these resistant strains is not significantly different from infections caused by more susceptible strains.[243] Pneumonia that is produced by relatively resistant pneumococcal strains may be treated with penicillin given in high doses.

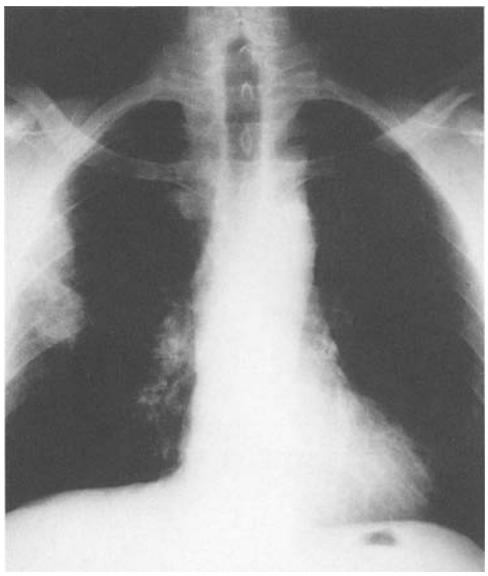
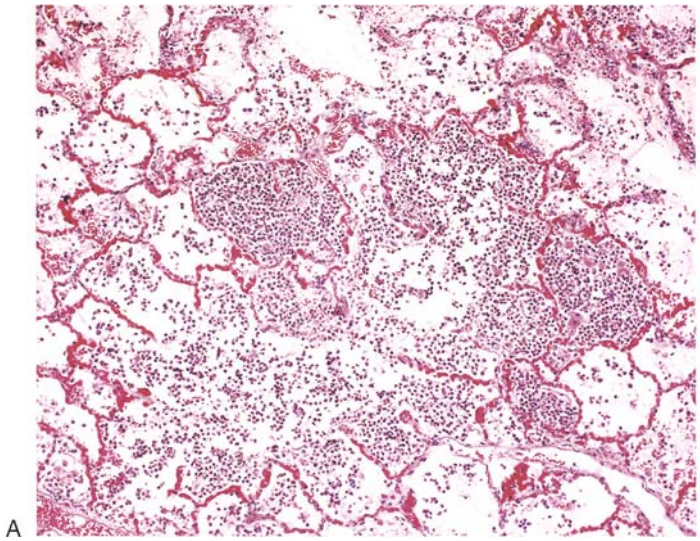


FIGURE 8.21. The focus of pneumonia is based on the pleural surface, suggesting the differential diagnosis of pulmonary embolus and hemorrhage. A rounded nodular outline is discernible within the larger infiltrate. *Streptococcus pneumoniae* type 3 was isolated in pure culture from an aspirate of the lesion.

FIGURE 8.20. Pneumococcal lobar pneumonia. The upper and middle lobes are densely consolidated, primarily in the stage of gray hepatization. The inflammation extends across the lobar fissures into the lower lobe, where there is an additional isolated focus (arrow).

The classic presentation of pneumococcal pneumonia is as a lobar consolidation (Figs. 8.10 and 8.20). It is important to recognize, however, that in a substantial proportion of cases the process is focal (Fig. 8.21). The inflammatory infiltrate consists of a mixture of polymorphonuclear neutrophils and macrophages (Fig. 8.22) surrounded by an edematous zone (Fig. 8.23). In the early stages of the infection pneumococci are easily demonstrated in the infiltrate (Fig. 8.24). An immunologically specific diagnosis can be made by reacting the bacteria with a polyvalent antiserum to capsular polysaccharide, the Quellung reaction.[244] This procedure works better on sputum smears (Fig. 8.25) or tissue imprints than on isolated bacteria. Although the Quellung reaction is useful for problem cases, the antiserum is very expensive and the information is not usually necessary.

Most nonfatal cases of pneumococcal pneumonia resolve without residua, but cavitary disease may occur,



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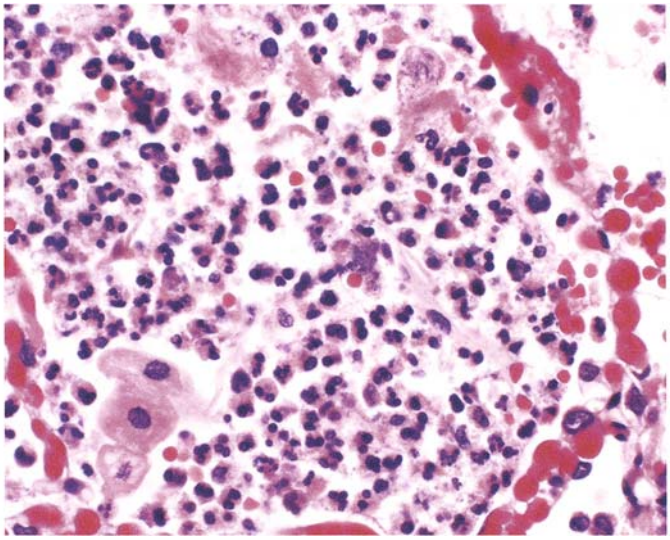


B

FIGURE 8.22. Pneumococcal pneumonia. **A.** Acute infectious pneumonia caused by *Streptococcus pneumoniae* is characterized by edema, neutrophils, and variable numbers of macrophages filling the air spaces. **B.** A higher magnification image nicely illustrates the constituent inflammatory elements.

particularly in patients who have bacteremic disease.[213,245] In some cases concomitant infection with bacteria that typically produce necrotizing infection may be responsible.[246] Respiratory distress syndrome as defined clinically has been described as a complication in fatal pneumococcal pneumonia.[247] Pneumatoceles, typically associated with staphylococcal pneumonia, have been reported in childhood pneumococcal infection.[248]

β-Hemolytic Streptococci

Streptococcus pyogenes (group A β-hemolytic streptococcus) pneumonia has been exceedingly rare and was uncommon even in the preantibiotic era.[202] The pneumonia may be secondary to streptococcal sepsis. The resurgence of virulent streptococcal infection in recent decades[249–252] may bode greater familiarity with this

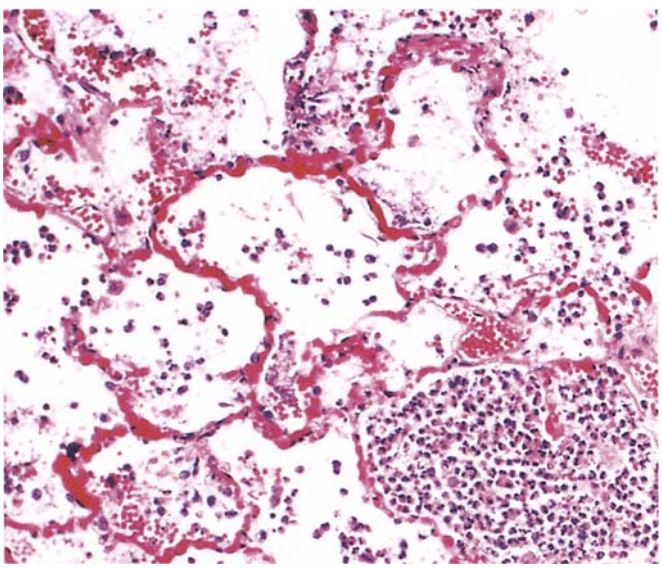


FIGURE 8.23. Pneumococcal pneumonia. At the periphery of the acute reaction edema and fibrin can be seen in the alveolar spaces.

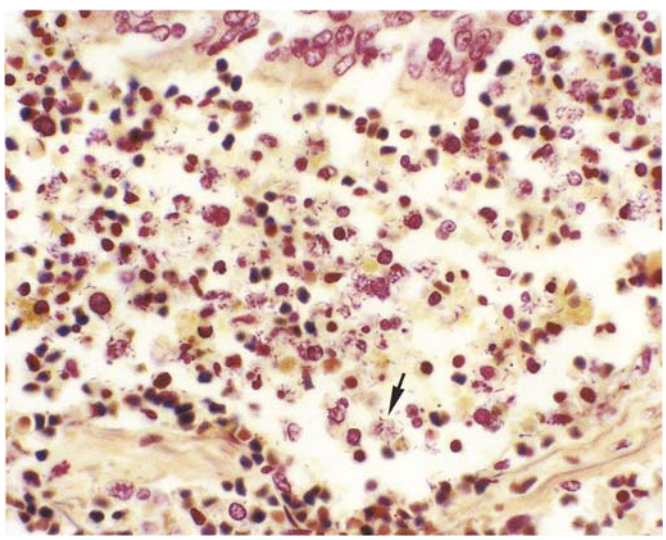


FIGURE 8.24. Pneumococcal pneumonia. A tissue Gram stain shows characteristic pneumococcal organisms in the acute infiltrate (Brown and Brenn). Many pneumococci are present here; a nicely preserved aggregate can be seen (arrow).

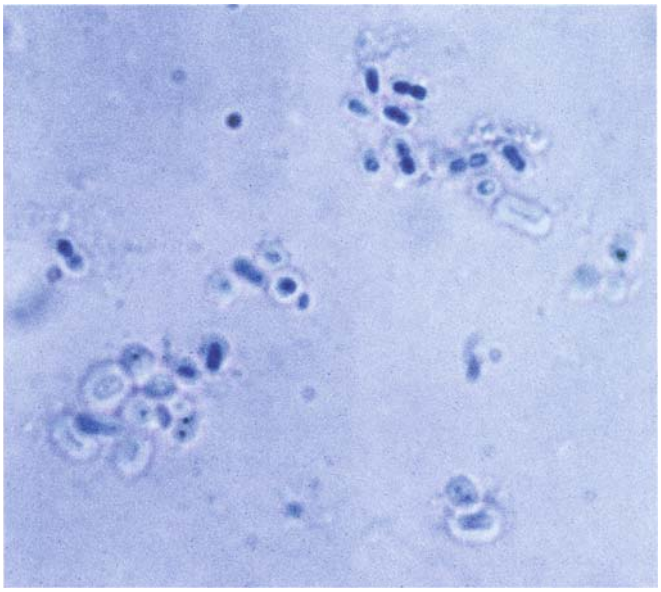


FIGURE 8.25. Pneumococcal pneumonia. Pneumococci in sputum specimen have been reacted with methylene blue and antiserum specific to *Streptococcus pneumoniae* type 3. Bacterial cells are stained by methylene blue; an abundant capsule is outlined by the precipitin reaction between the antibodies and the capsular polysaccharide.

awesome pathogen, which was the cause of the death of Jim Henson, the creator of the Muppets. In recent years, however, 10 to 15% of all invasive group A streptococcal infections have been associated with pulmonary disease.[251,252] Primary pulmonary infection often follows viral illness, particularly influenza,[126,253–255] but primary streptococcal pneumonia also occurs without antecedent disease.[255–258] Roy and coworkers[259] described a particularly virulent strain that caused symptomatic infection in seven of 12 family members, five of whom developed pneumonia. Their report suggested a precursor viral infection in two patients, although viral studies were incomplete, and no investigation into human leukocyte antigen (HLA) association was performed. Mortality, which was historically as high as 54%,[202] decreased dramatically with the introduction of penicillin therapy. Bacteremia is uncommon in primary *S. pyogenes* pneumonia.[255,256] Keefer and colleagues[255] detected bacteremia in 12% of 55 patients with primary streptococcal pneumonia; in those patients the mortality rate was 57% compared to 9% in the nonbacteremic patients. Although penicillin-resistant isolates have not yet been recognized, some of the virulent strains produce such rapidly fatal disease that antibiotics do not have a chance to kill the organisms.

The distribution of the lesions ranges from focal, lobular infiltrates, often confluent and extensive,[260,261] to lobar pneumonia. An interstitial pattern has also been described radiographically[253] and pathologically,[261] perhaps analogous to the familiar streptococcal cellulitis in the skin, but Goodpasture commented that the interstitial bronchopneumonia described by MacCallum as a sequel to measles was not present in his cases.[262] Macroscopically, Goodpasture[262] described the gray-purple color of the cut lung surface and the dry nature of the exudate in the bronchioles.

Early in the infection bacteria and inflammatory cells are scarce and hyaline membranes may be prominent.[262] At this early stage it may be difficult to distinguish between cases that were preceded clinically by a viral infection and those that were not, an observation that was later made by those who studied staphylococcal pneumonia in the influenza pandemic of 1957. Later, the infiltrate is rich in polymorphonuclear neutrophils, which may be extensively lysed by the bacterial enzymes, leaving a battlefield strewn with nuclear fragments and dead bacteria (Fig. 8.26). The result may be a very dusty-looking microscopic field at low power. Abscess formation is well described,[263] as is empyema[253] and bronchopleural fistula.[264] Kevy and Lowe[265] described empyema as a complication in 100% of their cases of group A streptococcal pneumonia.

Streptococcus agalactiae (group B β-hemolytic streptococcus) is best known as an important cause of neonatal pneumonia (see Chapter 7). There is a second peak of group B infections in the elderly, however[266,267]; the proportion of these cases with a pneumonic component ranges from 9% to 24%.[268,269] Verghese and colleagues[270] reported seven patients, two of whom had diabetes mellitus, which is increasingly recognized as a predisposing

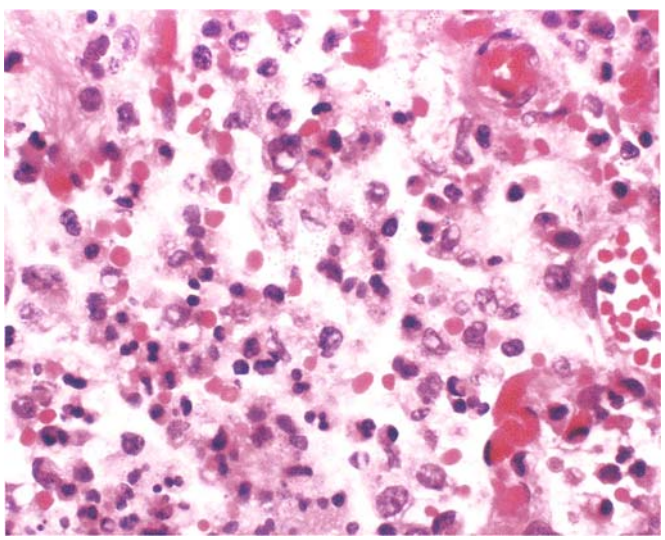


FIGURE 8.26. Streptococcal pneumonia. In the later phase of the acute pneumonia, the polymorphonuclear neutrophils may be extensively lysed by the bacterial enzymes, leaving a battlefield strewn with nuclear fragments and dead bacteria.

factor.[271–273] All seven patients died, but five of the seven cases were polymicrobial.

Group C β-hemolytic streptococci commonly produce disease in animals, including strangles in horses. They are generally recognized as etiologic agents of streptococcal pharyngitis and peritonsillar abscess[274–276] and may cause serious systemic disease.[277] Group C streptococci also may rarely produce serious pneumonia that resembles *S. pyogenes* infection in most parameters.[274,277–281] As in group A infections an interstitial pattern of infiltrates is often seen radiographically and empyema is a common complication.

Group C streptococci have been identified as *Streptococcus zooepidemicus* and *Streptococcus equisimilis* when appropriate biochemical studies are performed. These organisms are β-hemolytic on sheep blood agar, but may be γ-hemolytic on horse blood agar. Many strains are very susceptible to bacitracin and may be identified as group A streptococci if the taxonomic bacitracin disk is used for presumptive identification.[277]

These species rarely cause pneumonia and are more frequently found in the oropharynx of healthy individuals than are group A β-hemolytic streptococci, so that isolation from normally sterile sites, seroconversion, or critical analysis of laboratory and clinical data is necessary to document the isolate as an etiologic agent of the infection. Group C streptococci are susceptible to the action of penicillin, and resistant strains have not been described.

Other β-hemolytic streptococci rarely cause infection. A possible case of group G β-hemolytic streptococcal pneumonia in a newborn infant has been described.[277,282] Group D and F streptococci usually have an α- or γ-hemolytic reaction on blood agar and are discussed below.

Viridans Streptococci

Lancefield group F streptococci that are not β-hemolytic (α- or γ-hemolysis) have been recovered from serious lower respiratory infections.[222,283,284] These organisms are physiologically characterized variously as *Streptococcus milleri* in the British literature (not a taxonomically valid name) and *Streptococcus anginosus* in the American literature. They cause abscesses in many organs, including lung, liver, and brain. Approximately 20% of all infections caused by viridans streptococci involve the thorax.[285] In the lung they have been described as a component of polymicrobial lung abscess and as the sole cause of lung abscess. Empyema complicates a majority of these infections.[283] In one case the abscess was described as "foul smelling," a feature of anaerobic infection, but gram-positive cocci in chains were seen in smears and only *Streptococcus anginosus* was isolated. The possibility that *Peptostreptococcus* sp. was also present, but not isolated, cannot be eliminated. A case of empyema secondary to liver abscess without primary pneumonia has also been described.[286]

Sarkar and colleagues[287] described three cases of bacteremic pneumonia caused by *Streptococcus uberis.* The clinical course was uncomplicated, abscesses were not present, and the patients responded to penicillin therapy. Pratter and Irwin[288] reported a case of lung abscess and empyema caused by viridans streptococcus. The isolate was not completely identified, and the case may represent another example of *Streptococcus anginosus* infection.

Enterococcus spp.

Enterococci (formerly classified as streptococci) are rarely isolated from the oropharynx and the lower respiratory tract. Berk and colleagues[289] described a case of enterococcal pneumonia that developed after therapy with cephalosporins. The inflammatory response as observed in transtracheal aspirates consisted of polymorphonuclear neutrophils. One isolate was completely identified as *Enterococcus faecalis,* the most common enterococcal species. Enterococci are naturally resistant to cephalosporins and are recognized as potential problems in abdominal infections that are treated with these commonly used antibiotics. Optimal therapy is provided by the combination of a β-lactam antibiotic and an aminoglycoside (usually ampicillin and gentamicin). Strains that are intrinsically resistant to aminoglycosides and do not respond to combination therapy have been described with increasing frequency[290]; vancomycin alone or vancomycin and an aminoglycoside must then be used. Vancomycin-resistant strains have also been identified, leaving infectious disease physicians anxiously searching for alternatives.[291]

Staphylococcus aureus (Coagulase-Positive Staphylococcus)

Staphylococcus aureus is an uncommon but life-threatening cause of pneumonia both in the community and in hospitals; its incidence may be increasing.[292] Most patients who develop staphylococcal pneumonia have some underlying disease. Influenza virus infection is a frequent antecedent event when disease is community acquired. In a study of staphylococcal pneumonia, 52% of patients who were also tested for viral infection had either influenza A or B.[293] Staphylococcal pneumonia was a very frequent cause of secondary pneumonia in the 1957 influenza pandemic,[294] but has been less prominent in subsequent epidemics. Staphylococcal pneumonia also occurs in sporadic forms without regard to outbreaks of influenza virus, usually as a nosocomial infection.[214,295] In such cases the pneumonia may be primary, contracted through a respiratory route,[295] or may be secondary to infected foci in the viscera or soft tissues.[215]

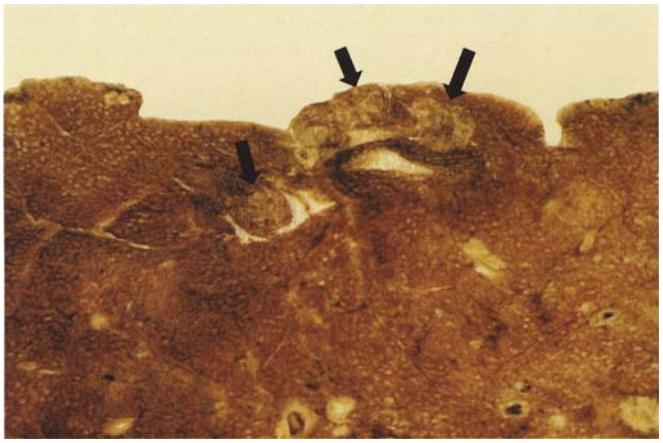


FIGURE 8.27. Mixed pneumonia caused by *Legionella pneumophila* and *Staphylococcus aureus.* Staphylococcal lesions in a paper-mounted whole-lung section are discrete, focal rounded densities that were microabscesses histologically (arrows). *Legionella pneumophila* and *Staphylococcus aureus* were isolated from postmortem lung.

Staphylococcal infections occur at the extremes of life.[191,199] Patients with coma[292] or on neurosurgical nursing units[296] are more likely to develop staphylococcal pneumonia. Patients infected with human immunodeficiency virus are also at increased risk. Levine and colleagues[141] identified *S. aureus* as the etiologic agent in seven of 102 patients who developed respiratory illness. It is noteworthy that *S. aureus* was isolated from respiratory secretions in 30 clinical episodes, but the bacteria were believed to be the etiologic agent in only eight instances. Even in severely immunosuppressed patients the isolation of most bacteria from potentially contaminated secretions does not establish an etiologic diagnosis. Finally, patients with cystic fibrosis are often colonized with *S. aureus* and may develop symptomatic infection of the lower respiratory tract. *Staphylococcus* usually is found in the early stages of disease, to be supplanted later by *P. aeruginosa.*[297]

The clinical presentation is quite variable. Patients are usually acutely ill; septicemic signs and symptoms may be dominant in disseminated infection. The radiographic distribution of the lesions may take any pattern, including lobar.[199] When the lungs are seeded from distant foci of infection, a metastatic pattern of multiple rounded densities may occur (Fig. 8.27).[215]

The inflammatory reaction is usually rich in polymorphonuclear leukocytes and bacteria are easily visualized with Gram stain (Fig. 8.28). Abscesses occur in 15% to 20% of patients,[214,293] but have been described in as many as 70% of primary pneumonias[295] and 80% of hematogenously derived infections.[215] Pneumatoceles, thin-walled abscesses visualized on chest radiographs, are classically described in children with staphylococcal pneumonia[199] but may occur after other bacterial infections as well.[298] Chartrand and McCracken[199] described pneumatoceles in 33 of 79 children (42%), usually by the fifth to the seventh day after admission (also see Chapter 7). Likewise, empyema is a recognized complication of staphylococcal pneumonia, occurring in 20% of adult cases[214] and as many as 75% of pediatric infections.[199,299] Atypical cases have been described in which a mixture of acute and chronic inflammation with fibrosis was present, perhaps influenced by partially effective antimicrobial chemotherapy.[300]

The mortality from staphylococcal pneumonia may be considerable. Bacteremia after primary infection develops in 25% to 40% of patients.[295,301] A rate of bacteremia as high as 89% has been reported when disseminated staphylococcal lesions are present; in this situation the bacteremia may persist even in the face of appropriate

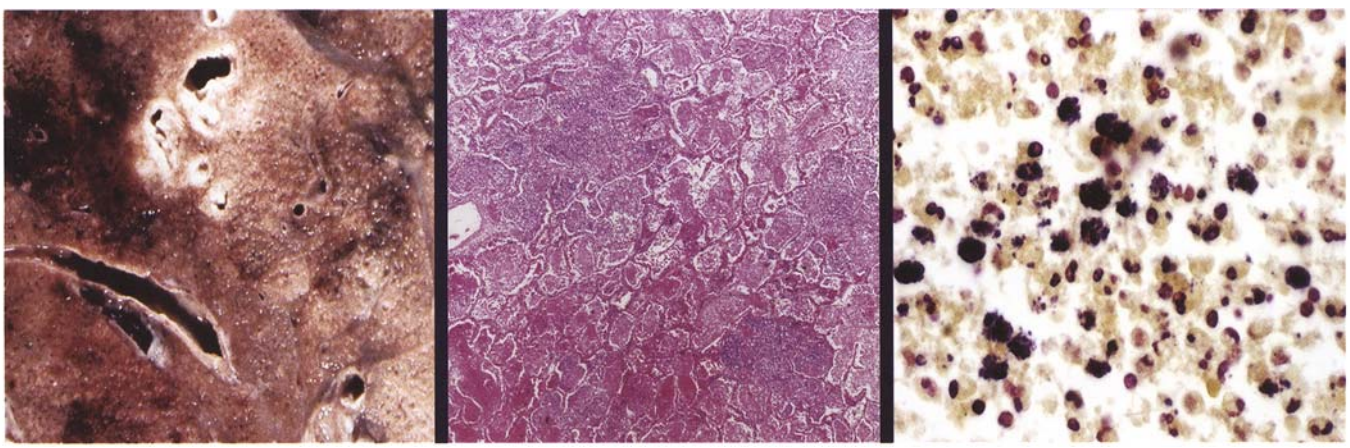


FIGURE 8.28. Staphylococcal pneumonia. The characteristic nodular configuration of staphylococcal pneumonia can be easily appreciated in both the gross **A.** and histopathologic **B.** appearance of this infection. **C.** A tissue Gram stain nicely demonstrates the gram-positive cocci of *Staphylococcus* (Brown and Brenn).

antimicrobial chemotherapy.[199] The death rate is commonly in the range of 25% to 30%,[199,214,293] but may be as high as 84% when bacteremia is present.[302] Therapy of the infections is complicated by the existence in many hospitals of bacterial strains that are resistant to multiple antibiotics, including the penicillinase-resistant penicillins and cephalosporins that are usually selected as therapy.[303] In these cases vancomycin is the only readily available alternative.

Other Gram-Positive Cocci

Souhami et al.[304] described a case of pneumonia in which *Micrococcus luteus* was isolated in heavy quantities and pure culture from a bronchial brush specimen. The patient, who had acute myelogenous leukemia and was leukopenic, developed cavitary disease but was successfully treated. The sputum was described as purulent, but the inflammatory nature of the material from the brushings was not mentioned. This organism is usually a saprophyte and is pathogenic in only rare instances.

Gram-Positive Bacilli

Nocardia *spp.*

Nocardiosis is an acute progressive or chronic bacterial infection caused by aerobic, exogenous, filamentous actinomycetes in the genus *Nocardia* and the order Actinomycetales.[305,306] The disease occurs worldwide and is often seen in persons who are immunocompromised or who have underlying medical conditions,[307–309] especially lymphoreticular malignancies,[310] granulocytopenia, chronic granulomatous disease of childhood,[311,312] and pulmonary alveolar proteinosis.[313–315] Most primary infections are pulmonary and result from inhalation of nocardiae that live as saprophytes in soil. Hematogenous dissemination from a primary pulmonary focus can involve almost any organ, but the brain, subcutaneous tissue, bones, joints, heart, and peritoneum are most often affected. The term *nocardiosis* refers to the disseminated disease in which nocardial filaments are randomly scattered within the invaded tissue. When nocardiae develop in the form of grains or granules in tissue, this rare localized form of the disease, usually seen in noncompromised patients, is classified as an actinomycotic mycetoma.

The three principal species that cause nocardiosis are *Nocardia asteroides, Nocardia brasiliensis,* and *Nocardia otitidiscaviarum.* Rarely, other species, such as *Nocardia transvalensis,* may produce similar disease.[316] Approximately 85% of nocardial infections are caused by *N. asteroides,* and this species is most often implicated in pulmonary infections. All three species are aerobic and easily cultured on Lowenstein Jensen medium at 30°C to 37°C. They will also grow, however, on blood agar and Sabouraud's agar that are antibiotic free. Colonies usually develop within 3 to 7 days, are heaped and folded, cream to yellowish-orange, and have a surface that is either moist and glabrous or covered with a powdery white aerial mycelium. The nocardiae are morphologically similar in cultures and clinical materials, appearing as delicate, branched filaments ≤1 µm in diameter. The filaments are often beaded, and fragmented bacillary and coccoid forms are occasionally seen. Isolates can be identified in culture by studying their physiologic and biochemical properties.[305,317,318]

Nocardiosis occurs three times as often in males as in females. It usually presents as either a chronic pneumonia in apparently immunocompetent individuals or as an acute, progressive pneumonia in immunodeficient patients.[319–326] Symptoms of pulmonary infection mimic those of tuberculosis and include fever, chills, dyspnea, cough, hemoptysis, chest pain, night sweats, and weight loss. Chest radiographs, which are nonspecific, usually reveal bilateral infiltrates and thin-walled cavities. Therapy consists of surgical drainage combined with high doses of trimethoprim-sulfamethoxazole or penicillins.[305,317,318,327]

The inflammatory response in nocardiosis is typically suppurative and necrotizing, leading to sinus tracts and encapsulated abscesses.[306] In chronic infections, multiple abscesses filled with thick, greenish-yellow, odorless pus are separated by areas of fibrosis (Fig. 8.29). The abscesses, which vary from 1 to 10 mm or more in diameter, are filled with neutrophils and macrophages. In chronic infections, epithelioid histiocytes and multinucleated giant cells are usually present at the periphery of the abscesses. The overall appearance of nocardiosis differs somewhat from actinomycosis in that the abscess cavities are less

FIGURE 8.29. Pulmonary nocardiosis. A peripheral focus of pneumonia includes several abscesses. *Nocardia asteroides* was recovered from the postmortem lung.

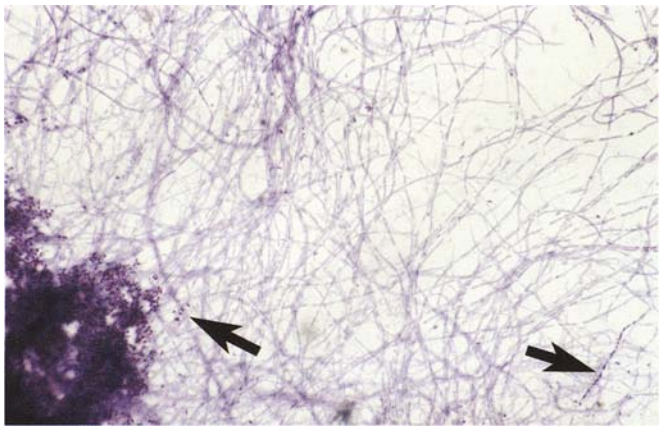


FIGURE 8.30. Pulmonary nocardiosis. A tangle of thin filaments are stained with methylene blue counterstain. Segments of the filaments stain red in this modified acid-fast stain (arrows). (Culture of material from a transbronchial biopsy; modified acid-fast stain.)

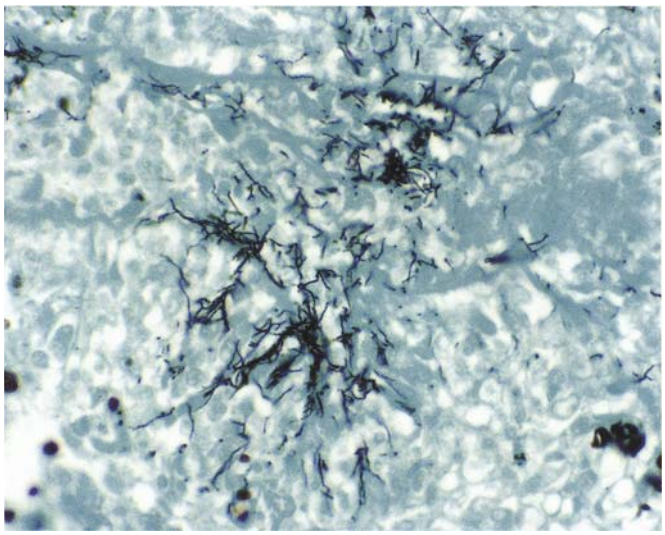
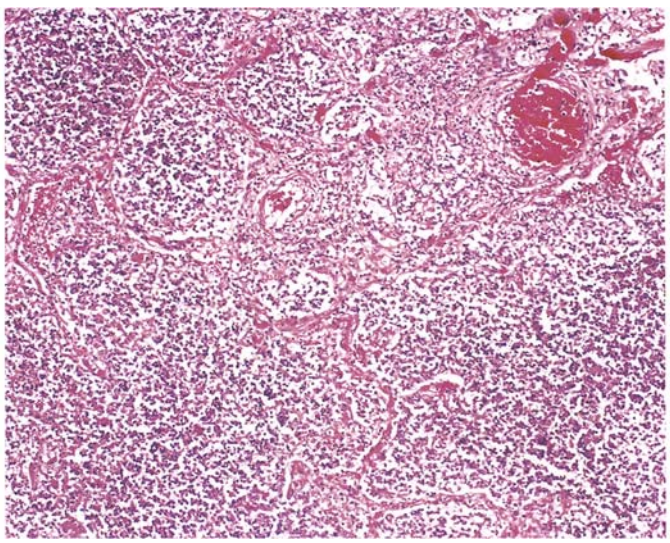


FIGURE 8.31. Nocardia. In tissue sections the nocardiae are readily demonstrated with the Grocott methenamine silver stain.

well defined and the fibrosis is less pronounced. Cavitation and pleuritis with empyema are frequent complications of pulmonary nocardiosis.[309,310,322,326]

In both acute and chronic lesions, individual nocardiae are diffusely distributed in the inflammatory exudate. The organisms appear as delicate, beaded filaments, ≤1 μm in width, that branch at predominantly right angles (Fig. 8.30).[306,328,329] In tissue sections the nocardiae are readily demonstrated with Grocott's methenamine silver stain (Fig. 8.31) and by a modified Gram's stain, such as Brown and Brenn or Brown-Hopps (Fig. 8.32). They are not reliably stained, however, with H&E, PAS, and Gridley fungus procedures. *Nocardia* spp. are weakly acid fast and nonalcohol fast when stained with modified acid-fast procedures that use an aqueous solution of a weak acid for decolorization.[306,329] It is important to note that some mycobacteria, predominantly rapid growers such as *Mycobacterium fortuitum* or *Mycobacterium chelonae*, are also partially acid-fast. Additionally, many bacteria, including mycobacteria, may be colored by the Grocott methenamine silver technique, especially if the staining time is prolonged.



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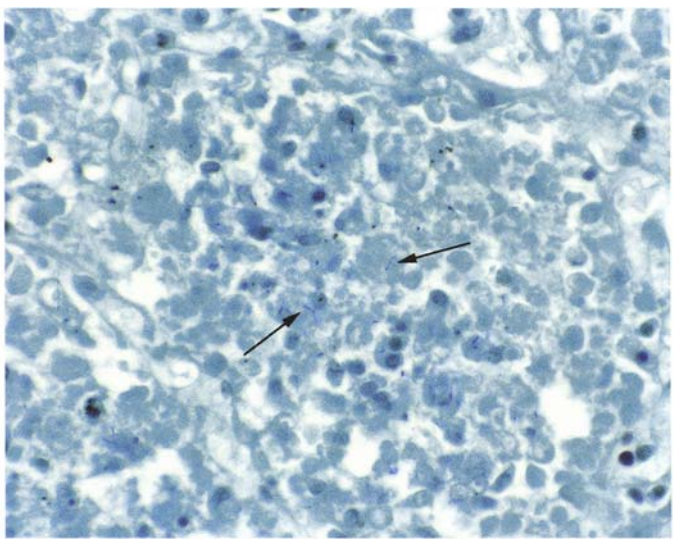

B

FIGURE 8.32. Nocardiosis. **A.** Acute necrotizing pneumonia caused by nocardia is characterized by purulent alveolar exudates. **B.** A Gram stain (Brown and Brenn) demonstrates thin filamentous gram-positive organisms (arrows). The filaments may break up, producing short bacilli or even coccobacilli.

Patients with pulmonary nocardiosis who are severely immunocompromised often present with progressive disease that appears as lobar, lobular, or fulminant necrotizing pneumonia.[306,309,324,325] Pulmonary fibrosis is minimal in these patients. The pneumonia is histologically similar to that caused by more commonly encountered bacteria. In the fulminant form of the disease, myriad nocardiae are often present and appear as faintly basophilic filaments and fragmented bacillary forms in H&E-stained sections. Large numbers of entangled nocardial filaments can form loose aggregates, but these aggregates do not resemble sulfur granules as seen in actinomycosis, nor are they surrounded by clublike Splendore-Hoeppli material.

An unusual association of nocardiosis is that with pulmonary alveolar proteinosis (see Chapter 21). The pathogenic relationship between these two entities is not completely understood.[313,314] *Nocardia* spp. have a predilection for causing pulmonary infection, and it is likely that alveolar proteinosis provides a favorable condition for growth of these actinomycetes. In addition to pulmonary nocardiosis, cerebral nocardiosis has also been reported in association with pulmonary alveolar proteinosis.[314]

Bacillus anthracis

Anthrax, historically referred to as "woolsorters' disease" in England,[330–332] is a highly lethal form of pneumonia and septicemia. Anthrax is caused by inhalation of spores of the large (1 µm in width by 300 µm to 1000 µm in length) gram-positive, nonmotile, toxin-producing rod *Bacillus anthracis*. This rare zoonotic disease is endemic in goats, sheep, cattle, horses, and pigs. It is usually spread to humans through the handling of contaminated hides, wool, hair, bone meal, or other animal products.[333] Those in close contact with these products or with animal tissues and fluids are at greatest risk. Infection occurs when spores of *B. anthracis* enter the body via percutaneous implantation, inhalation, or ingestion. The four major clinical forms of anthrax are cutaneous (malignant pustule), septicemic, pulmonary, and gastrointestinal.[19] The malignant pustule is the most common form and accounts for 95% of all infections.[19,334,335] Patients with pulmonary anthrax typically have no predisposing underlying illness, and the disease has a rapid onset characterized by fever, extreme weakness, nonproductive cough, severe dyspnea, tachycardia, and cyanosis. If untreated, anthrax is usually fatal within 4 days after the onset of symptoms. The diagnosis is seldom suspected, and the clinical course is usually very short.[336,337] *B. anthracis* is one of the prime targets for those who are preparing defenses against bioterrorism, because of its virulence, ease of cultivation, stability, and aerosolizable spore form. This bacterium has been implicated in at least two bioterrorist incidents.[21,338]

Human autopsy data from these events and experimental animal models of anthrax have shown that, after inhalation of spores, germination and multiplication of organisms occur in the tracheobronchial lymph nodes rather than in the lungs.[339–341] As a result, mediastinal tissues are profoundly affected, often demonstrating severe hemorrhagic edema and corresponding widening in chest radiographs.[338,341] A generalized septicemia follows germination. The lungs and other body sites are then involved by secondary spread, although direct retrograde extension from mediastinal structures and perihilar lymph nodes into peribronchial tissue has been suggested.[339] Vascular injury results from sepsis and individual toxin components.[20,342–345]

The most characteristic lesion of pulmonary anthrax is hemorrhagic edema (Fig. 8.33).[20,336,343] The lungs are heavy and have decreased crepitance. The pleural surfaces are smooth and there is usually a serosanguinous pleural effusion. The cut surfaces of the lungs are wet, and bloody fluid can easily be expressed from both bronchi and alveoli. Microscopically the lungs show massive hemorrhage and edema of all air spaces (Fig. 8.33). The most significant feature is the presence of a serofibrinous exudate and many large gram-positive bacilli in the absence of neutrophilic inflammatory exudate (Fig. 8.34). In the few patients who have a prolonged survival, septal necrosis may evolve and fibrin thrombi are present in alveolar capillaries.

Patients who are diagnosed as having anthrax before death should be autopsied with extreme care, because of the extreme hazards associated with handling *B. anthracis*. If tissue from suspected cases is available, the organism can be identified in impression smears or in fixed deparaffinized tissue sections by direct immunofluorescence (Fig. 8.35).[346]

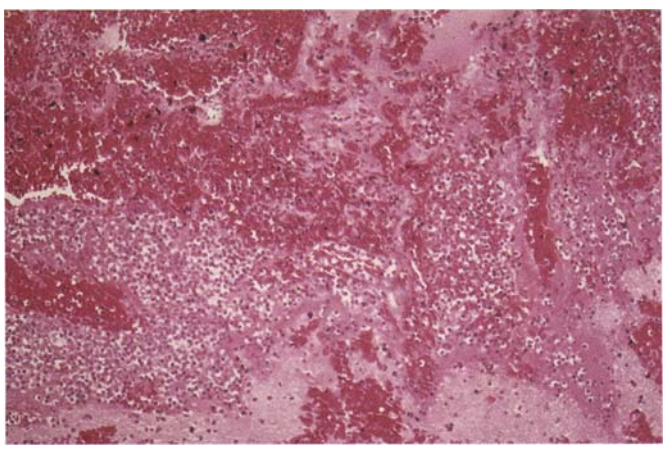

FIGURE 8.33. Pulmonary anthrax characterized by diffuse intraalveolar hemorrhage and edema. In this case there is also a neutrophilic infiltrate, an unusual finding in anthrax pneumonia.

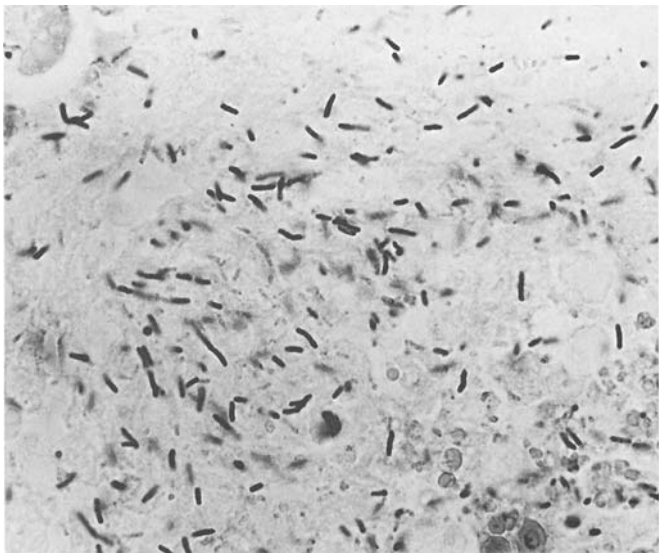

FIGURE 8.34. Pulmonary anthrax. Numerous large, elongated bacilli of *Bacillus anthracis* occupy a bronchiole and contiguous alveolar spaces. Note the absence of neutrophilic inflammatory exudate. (Steiner silver impregnation method.)

Rhodococcus equi *(Corynebacterium equi)*

Infection with the aerobic gram-positive opportunistic bacterium *Rhodococcus equi* (formerly *Corynebacterium equi*) is being reported with increased frequency in immunocompromised humans, particularly those with defective cell-mediated immunity.[347–354] Since *R. equi* was first reported to cause human infection in 1967,[355] more than 35 cases have appeared in the refereed literature.[349] Approximately one third of the infections have occurred in patients with AIDS or HIV infection. This bacterium has now been added to the list of opportunistic pathogens that define the acquired immunodeficiency syndrome.[349,356–358]

Rhodococcus equi is a well-recognized agent of respiratory and other infections in domestic animals, including horses, cattle, sheep, and swine. Foals are especially susceptible to infection and often develop suppurative pneumonia.[348,352,359,360] The bacterium is a common saprophyte of soil, which is believed to be the source for infections in animals.[359,360] Humans with *Rhodococcus equi* infections usually have a history of contact with farm animals or manure.[351,361] In patients who cannot recall animal contact, soil is believed to be the natural reservoir and source of the bacterium. In most instances, *R. equi* is probably acquired by inhalation of animal secretions or contaminated soil, after which there is a primary pulmonary infection.

The most common clinical manifestation of *R. equi* infection in humans is pneumonia, occurring in about 75% of all cases.[349] The onset is typically insidious with fever, cough, dyspnea, and fatigue. Chest radiographs often reveal a unilobar pulmonary infiltrate with no predilection for involvement of a particular lobe. After 2 to 3 weeks, the infiltrate may progress to involve several lobes or cavitate.[350,351,357,358] Pulmonary infections tend to be chronic, may mimic tuberculosis or other slowly progressive infections, may cavitate, and may produce pleural effusion or frank empyema.[362] The spectrum of pathologic findings includes acute suppurative bronchopneumonia (Fig. 8.36), necrotizing pneumonia with abscess

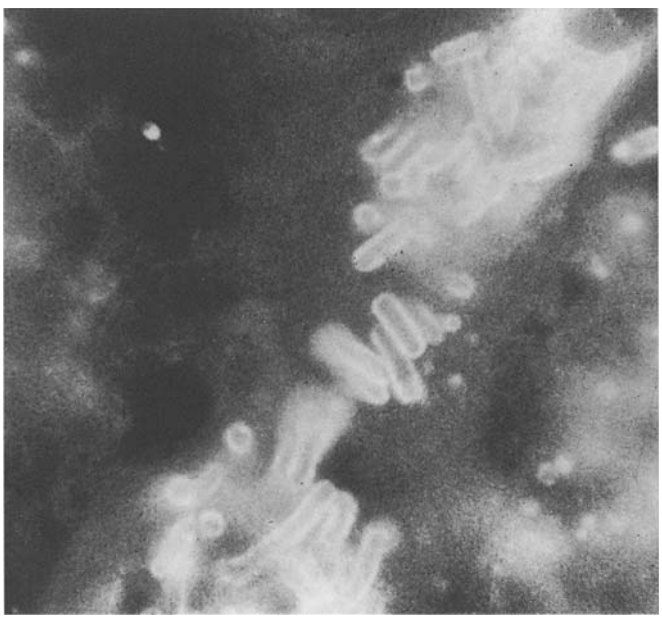

FIGURE 8.35. Immunohistologic detection of *Bacillus anthracis* uses fluorescein-labeled antiglobulins specific for this bacterium. Large, brightly fluorescent bacilli with rounded ends in terminal bronchiole.

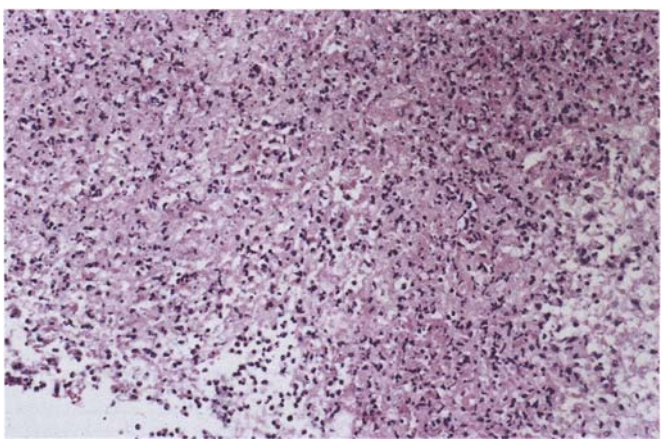

FIGURE 8.36. *Rhodococcus* pneumonia in a patient with AIDS. There is suppurative inflammation and necrosis with a minor macrophage component. *Rhodococcus equi* isolated from postmortem lung. (Case courtesy of A. Haque, University of Texas Medical Branch, Galveston, TX.)

formation and cavitation, and mixed suppurative and granulomatous pneumonia (Fig. 8.37) involving one or more lobes.[337,349,350,355,356] *R. equi* has also been reported to cause osteomyelitis, endophthalmitis, wound infection in a noncompromised individual,[363] and recurring bacteremia.[349,350,352]

Hematogenous dissemination from a primary pulmonary focus has been reported and *R. equi* has been isolated from several distant infected sites.[349,358] The mortality of disseminated *R. equi* infection is higher for patients who are infected with human immunodeficiency virus than for those who are not (55% versus 20%).[349]

In tissue sections there are foci of suppurative and granulomatous inflammation, which may resemble a malakoplakia-like eosinophilic histiocytic reaction (malakoplakia is discussed below). *R. equi* appears as numerous pleomorphic, gram-positive coccobacilli within macrophages and, less often, polymorphonuclear leukocytes (Fig. 8.38). The bacterium is easily delineated with modified Gram stains such as Brown and Brenn, and Grocott's methenamine silver stain. Most strains of *R. equi* are partially acid-fast with the Fite or Fite-Farraco stains, but they do not retain their acid-fastness when stained with the standard Ziehl-Neelsen procedure for mycobacteria. Partial acid-fastness can be an important clue to the identity of *Rhodococcus* spp. in tissue sections.[348,358]

In culture, most *R. equi* isolates of human origin are resistant to penicillins and cephalosporins. In vitro studies have shown that virulent strains of *R. equi* are resistant to phagocytosis and intracellular killing by macrophages.[364] It has been suggested, therefore, that antibiotics with two distinct characteristics, activity against *R. equi* in vitro and the ability to penetrate macrophages, should be selected for treatment.[349,362] Erythromycin and rifampin are two commonly used antibiotics that share these characteristics. Similar reasoning has been used to justify the efficacy of these two antibiotics against *Legionella* spp. in vivo. A prolonged course of antibiotics has been recommended because of frequent relapses. In addition to antibiotics, surgical extirpation or drainage of persistent, isolated lesions may also be beneficial.

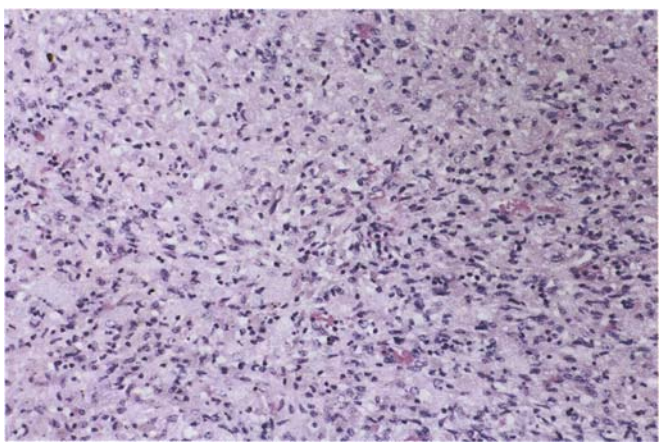

FIGURE 8.37. *Rhodococcus* pneumonia (same case as Fig. 8.38). Granulomatous inflammation predominates in this area of the pneumonic infiltrate.

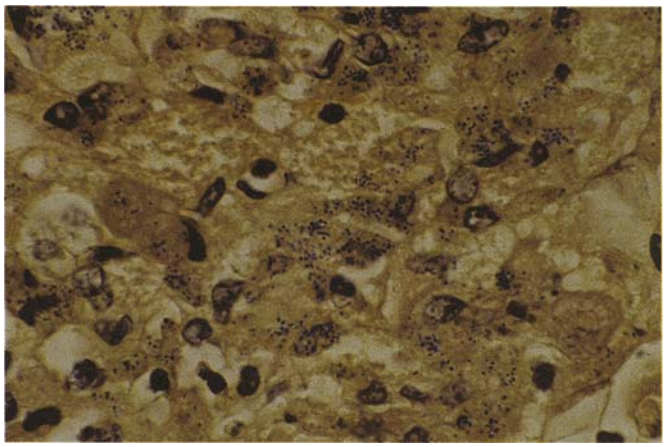

FIGURE 8.38. *Rhodococcus* pneumonia (same case as Fig. 8.37). Many gram-positive coccobacilli are present in the cytoplasm of some macrophages. (Brown and Hopps technique.)

Corynebacterium *spp.*

Several *Corynebacterium* species may produce pneumonia in immunosuppressed patients.

Corynebacterium jeikeium (formerly *Corynebacterium* group JK) is a species that produces septicemia in immunosuppressed patients, usually in the terminal stages of their illness. This species is found on the skin and may be introduced into the blood during the process of collection, so that routine identification of isolates is not useful. If multiple cultures from an immunosuppressed patient are positive, however, the isolate should be identified and tested for antimicrobial susceptibility, because this species is resistant to the commonly used β-lactam antibiotics. Pneumonia may result as a part of the septicemic process. Waters[365] described a case of *C. jeikeium* pneumonia in a neutropenic patient. The lung was edematous and hemorrhagic. Alveolar septa were focally necrotic and contained masses of proliferating bacteria (Figs. 8.39 and 8.40). Abscess cavities have been documented radiographically in pneumonia produced by this species.[366] Cases of pneumonia caused by *Corynebacterium pseudodiphtheriticum* and *Corynebacterium* group D2 have been reported in immunocompetent individuals.[367,368] A case of pneumonia attributed to *Corynebacterium pseudotuberculosis*, ordinarily an animal pathogen, has been described in a veterinary medical student.[369] A transtracheal

FIGURE 8.39. *Corynebacterium jeikeium* pneumonia in a severely immunocompromised, neutropenic patient. A large consolidated necrotic gray nodule is surrounded by a hemorrhagic lung. Note lobular demarcation of the lesion. (Courtesy of Dr. B. Waters.)

aspirate from which the bacterium was isolated contained polymorphonuclear neutrophils, but a transbronchial biopsy demonstrated interstitial fibrosis and intraalveolar eosinophils.

Miscellaneous Gram-Positive Bacilli

Listeria monocytogenes is a well-recognized cause of intrauterine and neonatal infection, as well as meningitis in adults. In patients who are infected with HIV, this bacterium produces a greater variety of infections, including brain abscess.[370] Pneumonia is a rare manifestation of *Listeria* infection. Whitelock-Jones and colleagues[371] reported an unusual case of cavitating pneumonia in a previously healthy man. *L. monocytogenes* was isolated from blood.

Bacillus cereus may cause serious infections, including pneumonia, especially in patients who are immunosuppressed.[372] Bekemeyer and Zimmerman[373] reported an 18-year-old, previously healthy, man who developed massive hemoptysis, bronchopleural fistula, and empyema. *B. cereus* was isolated from sputum and in pure culture from pleural fluid.

Lactobacillus spp. are common commensals in the vagina and in the upper respiratory tract. This genus is difficult to identify, and differentiation from streptococci may even be a problem occasionally. Rarely *Lactobacillus* spp. may produce serious infection, including pneumonia. Querol and colleagues[374] reported a cavitating lesion in a 40-year-old heavy smoker and drinker who developed foul-smelling sputum, usually associated

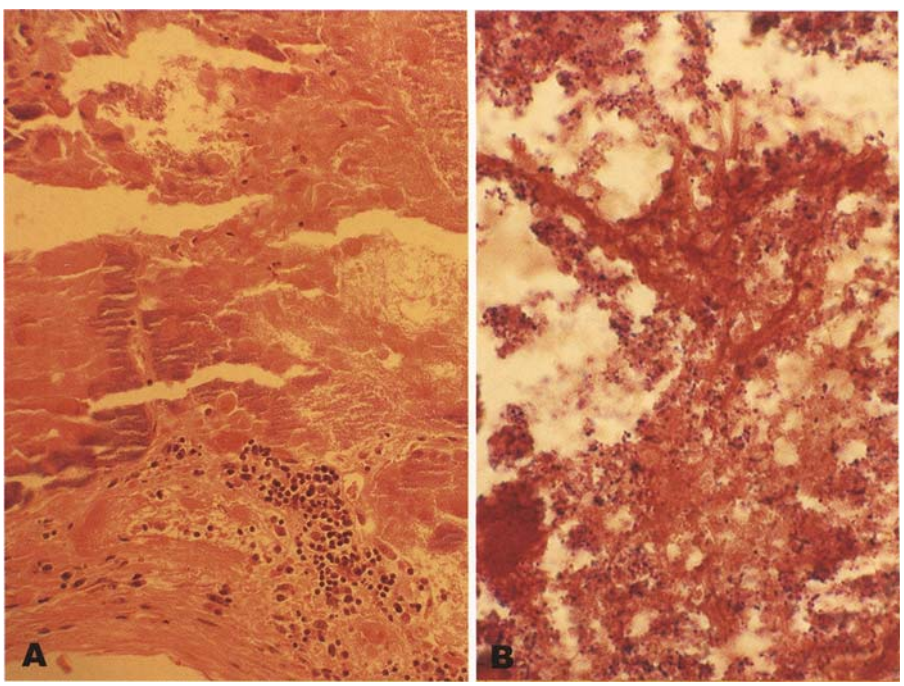

FIGURE 8.40. *Corynebacterium jeikeium* pneumonia. **A.** Lymphocytes are present in the adventitia of a pulmonary blood vessel from the case depicted in Figure 8.39, but a fibrinous exudate with no acute inflammatory cells is present in the air spaces. **B.** Numerous small gram-positive coccobacilli in the exudate are demonstrated by a tissue Gram stain. The differential diagnosis includes other "diphtheroids" and elongated streptococci. (A, hematoxylin and eosin [H&E]; B, Brown–Hopps procedure.)

with anaerobic infection. *Lactobacillus* sp. was isolated from blood and from the lung in pure culture by a needle aspirate that also contained many neutrophils. We have observed a similar patient at the Medical Center Hospital of Vermont who was infected with a strain of *Lactobacillus* sp. that was resistant to vancomycin, an antibiotic that is very effective against most gram-positive organisms.[375]

Rothia dentocariosa is a gram-positive coccobacillus that resembles *Corynebacterium* and normally inhabits the oral cavity. On rare occasion this bacterium may produce severe infection, such as endocarditis. Schiff and Kaplan[376] have described an acute upper lobe pneumonia in an 84-year-old woman with acute myelocytic leukemia. *Rothia* was isolated in pure culture from a lung aspirate, but the inflammatory reaction was not described. This organism can also produce a pathologic process that resembles actinomycosis (see below).

Gram-Negative Cocci

Neisseria meningitidis

Neisseria meningitidis is a well-established pathogen that usually causes meningitis or septicemia, but also colonizes the upper respiratory tract of many individuals. Colonization is not a risk factor for subsequent disease in nonepidemic situations. Pneumonia is an uncommon manifestation of *N. meningitidis* infection, although the true incidence is difficult to determine.[377] This infection is frequently preceded by a viral upper respiratory infection, but may also be one of the bacterial complications of measles.[378] The source of the bacterial inoculum is presumably the upper respiratory tract. Most infections are community acquired, but nosocomial meningococcal pneumonia has also been described.[379] The pathogenesis of meningococcal pneumonia may be aspiration of indigenous flora, even in the hospital setting, but Rose and colleagues[380] have reported two cases of pneumonia caused by *N. meningitidis* serogroup B in which transmission of bacteria from one patient to another may have been accomplished by hospital staff.

Most cases of meningococcal pneumonia, in which typing of the bacterial isolates has been done, are caused by serogroup Y,[378,381–383] but serogroup W-135[384,385] and serogroup B[380] have also been reported. Meningococci may be isolated from sputum in the absence of lower respiratory tract disease. If the isolate is typed as serogroup Y, there is a greater association with pneumonia, but careful correlation with clinical data must be done before accepting the isolate as the etiologic agent of the infection.

Most cases of meningococcal pneumonia appear to be focally distributed. A lobar radiographic pattern has been described in one case.[383] The clinical course is usually uncomplicated, although bacteremia may occur,[385] and empyema may rarely ensue.[386]

Other Neisseria *spp.*

Neisseria gonorrhoeae ordinarily is found in the respiratory tract only in the pharynx, where it can produce acute pharyngitis.[387] An unusual case of pneumonia complicated by empyema has been attributed to *N. gonorrhoeae.*[200]

Other *Neisseria* species are common members of the indigenous upper respiratory tract flora. They may be isolated from polymicrobial infections, but are unusual sole isolates from cases of pneumonia. *Neisseria cinerea* has been reported as the etiologic agent of a case of nosocomial pneumonia.[388] *Neisseria sicca*, frequently present in the upper airway, has caused pneumonia in patients who had bronchiectasis,[389] were pregnant, or had bullous pemphigoid that was treated with steroids.[390] *Moraxella catarrhalis*, which was formerly classified as *Neisseria catarrhalis* and later as *Branhamella catarrhalis*, is discussed with the gram-negative bacilli.

Gram-Negative Bacilli

Enteric Gram-Negative Bacilli

The enteric gram-negative bacilli constitute a large group of organisms in the family Enterobacteriaceae. They are genetically related and share phenotypic characteristics that allow microbiologists to classify them. They are relatively plump bacilli, which may sometimes be coccobacillary but without the pleomorphism of *Haemophilus* spp. Biochemically they are oxidase negative, ferment glucose, and reduce nitrate to nitrite. In recent years, as the geneticists have worked their craft, the number of species in the Enterobacteriaceae has increased but the familiar names still predominate. Virtually any of these genera may produce pneumonia, usually in a nosocomial setting. The most venerable pathogen historically is *K. pneumoniae;* a closely related species, *Klebsiella oxytoca*, has recently been differentiated taxonomically.[37]

The enteric gram-negative bacilli have many epidemiologic and clinical characteristics in common. They usually produce pneumonia in patients who have underlying conditions, such as alcoholism, diabetes mellitus, chronic obstructive lung disease, or immunocompromised status by virtue of underlying disease or immunosuppressive chemotherapy.[134,391–396] The pathogenetic sequence usually includes colonization of the oropharynx and aspiration of secretions. Abscess formation and empyema occur more frequently than in pneumococcal pneumonia. Bacteremia varies by species and is associated with poor prognosis, including a mortality rate of 50% to 100%.[393] *Serratia* spp. tend to produce bacteremic pneumonia, whereas *E. coli* and *K. pneumoniae* cause nonbacteremic infections.[393]

Klebsiella pneumoniae

Klebsiella pneumoniae was first isolated and associated with pneumonia by Friedländer[397] in the 19th century. Some strains of the bacterium have a prominent polysaccharide capsule analogous to that of *S. pneumoniae.* The capsule can be demonstrated in clinical material with the Quellung reaction. Zander[398] described an epidemic of *Klebsiella* pneumonia in which the lesion was primarily lobular pneumonia. In the series of 14 South African patients reported by Erasmus, 13 individuals had radiographic evidence of consolidation in portions of one lobe, most commonly of the right lung, or diffuse patchy infiltrates.[399] Lobar involvement has also been described (see Fig. 8.11),[400–402] and a bulging fissure caused by expansion of the lobar volume has been considered radiographically characteristic.[403,404] Most of the cases described radiographically and pathologically by Bullowa and associates[402] had a lobar distribution. The consolidation often extended across the lobar fissure to involve adjacent lung. Fremmel and colleagues,[405] however, postulated that the primary pathologic process was focal and that progressive extension of the infiltrate produced a pseudolobar pattern. As has been mentioned previously, the differentiation of lobar from confluent focal pneumonia may be difficult pathologically and is probably impossible radiographically. Unger and colleagues[403] noted massive consolidation in a majority of patients.

Klebsiella pneumoniae produces acute infection in the community, usually in persons who are at increased risk of infection, most commonly because of alcoholism. It is also an important nosocomial pathogen in modern hospitals and may be resistant to multiple antibiotics.[406] The sputum has been described as blood-tinged or rusty, resembling that found in pneumococcal lobar pneumonia. In approximately one third of the cases described by Bullowa and colleagues[402] the expectorated sputum had a distinctive thick, gelatinous, diffusely bloody appearance. The inflammatory process is usually neutrophilic and necrotizing (Fig. 8.41). Gram-negative bacilli are readily demonstrated in tissue or respiratory secretions (Fig. 8.42). Unger and colleagues[403] noted abscesses radiographically in seven of 15 patients, in two of whom the cavities were massive. Abscesses were present in two thirds of the cases reported by Belk.[400] In one instance the destructive process was sufficiently extensive to warrant the designation of gangrene.[216]

Chronic pneumonia has also been attributed to *K. pneumoniae.* The course of these unusual cases is subacute or chronic and may be confused clinically with tuberculosis.[407,408]

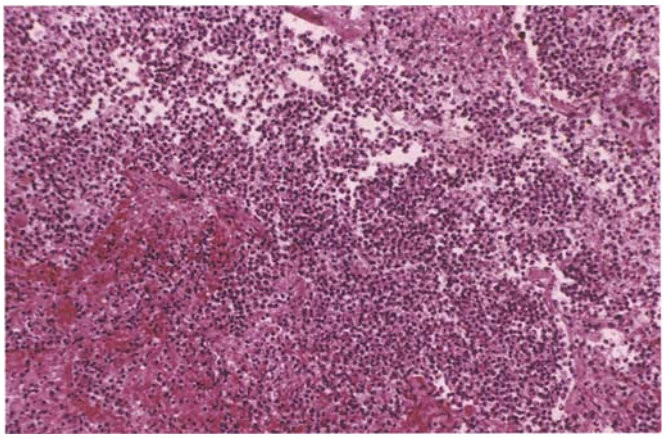

FIGURE 8.41. *Klebsiella pneumoniae.* Mixed inflammatory exudate and fibrin fills air spaces; alveolar septa have been disrupted.

Escherichia coli

In 1967, Tillotson and Lerner[409] described the characteristics of 20 cases of pneumonia caused by *E. coli.* All patients had serious underlying diseases, predominantly diabetes mellitus. Focal pneumonia (Fig. 8.43) was seen radiographically and was present in all seven patients who were autopsied. Eight of 18 patients tested were bacteremic, a finding that indicated a worsened prognosis. Empyema, which did not affect the prognosis, was noted in eight patients. Microscopically, pulmonary hemorrhage was noted in two patients who died within 48 hours of onset, but neither vascular inflammation nor thrombosis was seen. Later in the process the air-space infiltrate was primarily mononuclear, except in areas of necrotizing inflammation where polymorphonuclear neutrophils predominated. Macroscopic abscesses were noted in two patients.

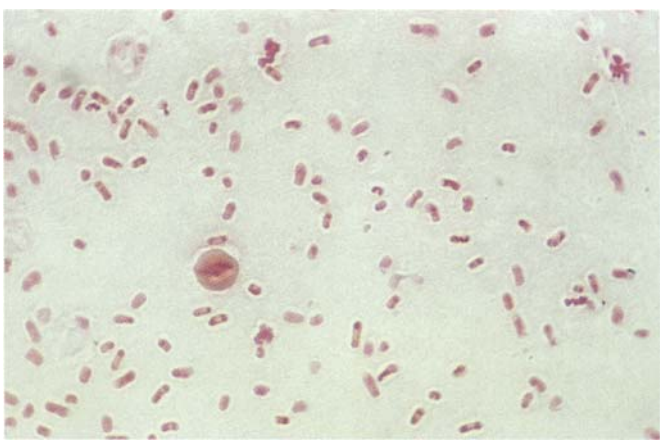

FIGURE 8.42. *Klebsiella pneumoniae.* Gram stain of respiratory secretions from case pictured in Figure 8.11 shows macrophage surrounded by many plump gram-negative bacilli. The halo around the bacteria, suggesting a capsule, is not definitive evidence for encapsulation. (Gram stain.)

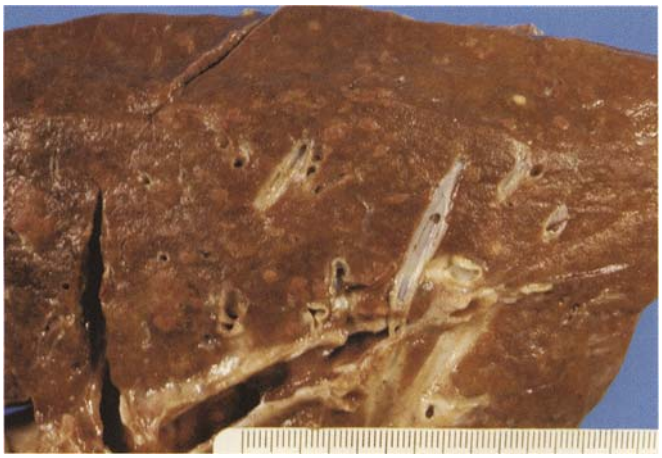

FIGURE 8.43. *Escherichia coli* pneumonia. Multiple focal tan nodules present in the lung of the patient. *Escherichia coli* was isolated from the blood antemortem.

The prevalence of *E. coli* pneumonia in the elderly has been emphasized by several authors.[410,411] The pulmonary infiltrates were described radiographically as patchy; dense consolidation occurred in only a minority. Berk and colleagues,[411] who examined the isolates for the K1 capsular polysaccharide antigen, found six of 17 strains with this characteristic, which has been associated with neonatal infections. Sputum contained the causative organism in most of the bacteremic cases.[409–411]

Enterobacter *spp.*

Enterobacter spp. have been reported infrequently as etiologic agents of pneumonia,[394,396,403,412] although they are important nosocomial pathogens. This genus has been particularly adept at developing broad resistance to β-lactam antibiotics after exposure to third-generation cephalosporins.[413] The available evidence suggests that the spectrum of pulmonary disease produced by *Enterobacter* spp. resembles that of *K. pneumoniae.*

Serratia *spp.*

Serratia marcescens is the most important pathogen in the genus. This bacterium has a venerable and colorful history, often related to the bright red pigment that many isolates produce. Raphael's fresco, *The Mass of Bolsensa*, commemorates the miraculous appearance of blood on the Eucharistic wafer, now interpreted as contamination of the bread by *S. marcescens.* This organism was once considered a harmless commensal, to be swabbed on the hands of medical students in epidemiology demonstrations or released into the environment as experiments in biological warfare.[414] Outbreaks of nosocomial infection were associated with some of the military aerosol experiments, but retrospective analysis has suggested that the associations were fortuitous. Rather, the outbreaks called attention to nosocomial infections that had not been recognized previously. *S. marcescens* may cause infections as a member of the patient's indigenous flora, after transfer on the hands of medical personnel or through contamination of therapeutic or diagnostic reagents.[415] Most patients have serious underlying disease. The etiologic agent is usually isolated from sputum, where it must be differentiated from colonizing flora. A minority of patients develop bacteremia. *S. marcescens* was recovered only at postmortem examination in seven of 40 patients reported by Goldstein and colleagues.[217]

The radiographic[416] and pathologic[217] features of nosocomial *Serratia* pneumonia at one institution have been described. Goldstein and associates[217] reviewed the pathologic findings in 40 cases of pneumonia in which *S. marcescens* was isolated, including 16 cases in which the bacterium was isolated in pure culture. The pathologic response differed in patients who were neutropenic and those in whom the circulating neutrophils were intact. The most common macroscopic appearance was focal consolidation and focal hemorrhage, which occasionally became confluent. The radiographic analysis of cases from this institution included two cases in which the distribution was described as lobar, perhaps reflecting the difficulty of differentiating confluent focal pneumonia from lobar consolidation radiographically.[416] In the neutropenic patients the lungs were diffusely edematous and hemorrhagic.

Microscopically, the inflammatory exudate in nonneutropenic patients consisted of polymorphonuclear neutrophils, macrophages, fibrin, and hemorrhage. Necrotizing inflammation was common, as were microabscesses or macroscopically and radiographically visible cavities. Gram-negative bacilli were readily demonstrable in the exudate. In seven of nine cases there was a distinctive vasculitis of arteries and veins. Polymorphonuclear neutrophils infiltrated the intima and media, and gram-negative bacilli were demonstrated intramurally in two cases. Vascular thrombosis and necrosis were not observed. The authors noted the similarity of this vasculitis to that found in *Legionella* pneumonia and the differences from the lesions found in *Pseudomonas* infections.[217]

The inflammatory exudate in the neutropenic patients included fibrin, hemorrhage, and hyaline membranes. In some cases bacteria were present without cellular reaction; in others macrophages were increased in number.

The lungs of four patients with *S. marcescens* pneumonia contained intraalveolar inflammatory organization or bronchiolitis obliterans in addition to necrotizing or neutrophilic pneumonia.[217] Carlon and associates[417] reported a patient who developed interstitial fibrosis, as

demonstrated by chest radiographs, after an acute *Serratia* pneumonia.

Proteus *spp. and Related Organisms*

The tribe Proteeae consists of the genera *Proteus, Providencia*, and *Morganella*, which are infrequent causes of pneumonia. Walter Reed[418] reported an early case in which *Proteus vulgaris* was isolated from lung and from sputum by inoculation of rabbits. Lancet-shaped diplococci were also present, although not isolated in either culture, so the role of *Proteus* in the infection is unclear. Tillotson and Lerner[419] described six cases caused by *P. mirabilis, P. vulgaris*, and *P. morganii* (currently classified as *Morganella morganii*). All patients had chronic lung disease and five were alcoholic. The episodes of pneumonia were preceded by episodes of decreased consciousness, and the resultant pneumonia had a lobar appearance radiographically. Microscopically, there was a mixed mononuclear and polymorphonuclear inflammatory cell infiltrate. Abscesses were present in five of six cases. Empyema was not observed in this series, but has been reported. Lysy and colleagues[420] described pneumatoceles, large air-filled spaces usually associated with staphylococcal pneumonia, in an infection produced by *P. mirabilis.* Pneumatoceles are produced by destruction of lung tissue, but are thin walled in comparison to chronic abscesses, are usually visualized radiographically, and ordinarily resolve without clinically evident residua. *Proteus* lobar pneumonia has also been reported in a previously healthy adult.[421] Focal pneumonia with abscess formation has also been associated with infection by *Providencia* species, but detailed descriptions of the pathology are not available.[422]

Salmonella *spp.*

Cough and pulmonary infiltrates are a regular part of the typhoid fever syndrome,[423–425] but *Salmonella* species are not usually considered primary pulmonary pathogens. Aguado and colleagues[426] described eight patients with pleuropulmonary infections caused by nontyphoidal *Salmonella* species; *Salmonella* was isolated from the stool of only two patients. Serious underlying disease was present in all patients, and seven of 11 were immunosuppressed. Eight patients had focal pneumonia, two had discrete lung abscesses, and one had an empyema. *Salmonella* pneumonia has been described as a complication of neoplastic disease, including carcinoma[427] and lymphoma.[428]

Yersinia pestis

Plague is caused by the small, gram-negative, nonmotile, and non–spore-forming coccobacillus, *Yersinia pestis.* This organism, which was formerly named *Pasteurella pestis*, was transferred into the newly created genus *Yersinia*, a member of the family Enterobacteriaceae, on the basis of biochemical and genetic relatedness.[37] At the same time, *Pasteurella tularensis* was placed in the newly created genus *Francisella.*

Plague, which is a disease of both historic and current interest, can exist in either the bubonic, primary septicemic, or pneumonic forms. Clinical disease results from the rapid, uncontrolled multiplication of plague bacilli in infected tissues and the subsequent production of two major toxins—endotoxin and murine toxin.[429–431] The gross and microscopic appearances of the lungs are similar in all forms of the disease.

Bubonic plague, which decimated the population of Western Europe during the Middle Ages, has been referred to as the "black death." The environmental disease reservoirs are wild rodents, especially rats and mice in urban environments, and ground squirrels, chipmunks, and prairie dogs in the southwestern United States. The disease is transmitted to humans either by direct contact or by the bite of a rodent flea.[432,433] Following entry of the organism, the regional lymph nodes become infected. After a few days the lymph nodes may enlarge to form the characteristic painful fluctuant buboes of bubonic plague.[434] If bacteremia develops by release of bacteria from the buboes, the lungs as well as the spleen, liver, meninges, and other body sites may become secondarily infected via hematogenous dissemination. In primary septicemic plague, patients have minimal or no lymphadenopathy, and organisms pass rapidly through regional lymph nodes to the vascular system. In either bubonic or primary septicemic plague, patients with pulmonary infection typically produce large amounts of bloody or frothy sputum containing myriad bacilli. They readily disseminate bacteria into their environment by the aerosol route.

Pneumonic plague, or primary plague pneumonia, is the most rapidly lethal form and results from the inhalation of aerosolized droplets of infected secretions. Historically, pneumonic plague has been much less common than bubonic or septicemic plague, although occasional cases have been seen during outbreaks of bubonic disease. The only well-investigated epidemic of primary pneumonic plague occurred in Manchuria during the early 20th century.[435] In this outbreak the disease apparently spread rapidly from person to person among those living in poorly developed, cold, crowded areas. Although the disease probably originated in rodents, rapid and efficient transmission no longer required an insect vector after the airborne route of spread evolved.

Sporadic cases of pneumonic plague also occur. In the western part of the United States these cases are usually associated directly or indirectly with endemic foci of disease in wildlife. Werner and colleagues[436] reported a

fatal case of primary plague pneumonia in a woman who contracted the disease from a cat that also had pneumonic disease. Another case illustrates the transient nature of the contact necessary to produce fatal pneumonic infection: A 31-year-old man died of primary *Yersinia pestis* pneumonia after he removed an infected domesticated cat from the crawl space under a house in Colorado.[437] The cat, which had oral and submandibular lesions compatible with feline plague, probably was infected after contact with endemically infected chipmunks in the area. In this case the correct diagnosis was delayed because a packaged microbiology system, which did not contain *Yersinia pestis* in its database, incorrectly identified the sputum isolates as *Yersinia pseudotuberculosis.*

Primary plague pneumonia is characterized by widespread hemorrhagic lobular lesions that may become confluent over large areas of the lung to produce a lobar and then multilobar pneumonia.[438] Fluid can be expressed readily from cut surfaces. Peribronchial and mediastinal lymph nodes are enlarged and may be edematous and hemorrhagic.

Microscopically, the inflammatory exudate is characterized by hemorrhage and edema; fibrin is almost always absent (Fig. 8.44). Macrophages and scant neutrophils are present within the alveoli, and there is extensive parenchymal necrosis. Massive numbers of gram-negative, bipolar-staining coccobacilli that measure 0.5 to 1.0 μm by 1.0 to 2.0 μm are present in bronchi, bronchioles, and alveoli (Fig. 8.45). The organisms are best demonstrated with Gram stain of impression smears. In tissue, the Brown–Hopps tissue Gram stain and silver impregnation stains, such as the Steiner, Warthin–Starry, or Dieterle procedures, are preferred. Because bipolar staining is typically present, *Y. pestis* has been described as having a safety-pin appearance. This bipolar staining is best demonstrated in methylene blue or gram-stained impression smears; such structural detail is lost when the silver impregnation stains are employed. In pneumonic plague secondary to septicemia, the inflammatory response is similar to that seen in primary plague pneumonia. In septicemic disease, however, plague bacilli associated with parenchymal necrosis are often more numerous in the interstitium than in the alveoli.[439] Extreme caution should be taken when caring for plague patients, when handling autopsy and surgical specimens, and when handling the organism in the laboratory. The disease can be acquired by the respiratory route, and laboratory-acquired cases have been reported.[438] It is frequently desirable to identify *Y. pestis* in smears of respiratory secretions or in formalin-fixed deparaffinized tissue sections by direct immunofluorescence (Fig. 8.46), because of the risks associated with handling cultures of the organism.[346]

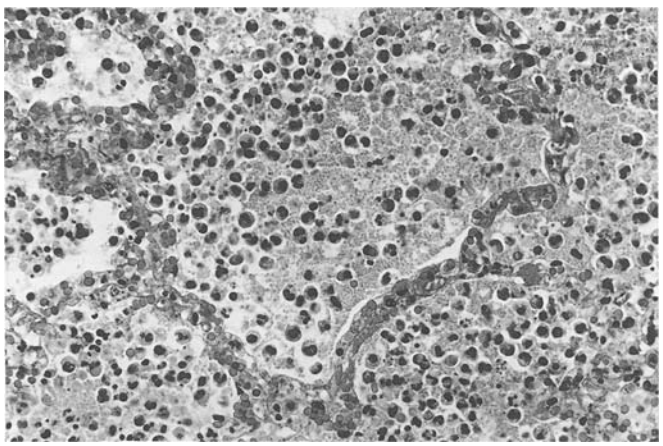

FIGURE 8.44. Pneumonic plague. Alveolar spaces contain compact masses of poorly stained coccobacilli mixed with fewer neutrophils and macrophages; fibrin is absent.

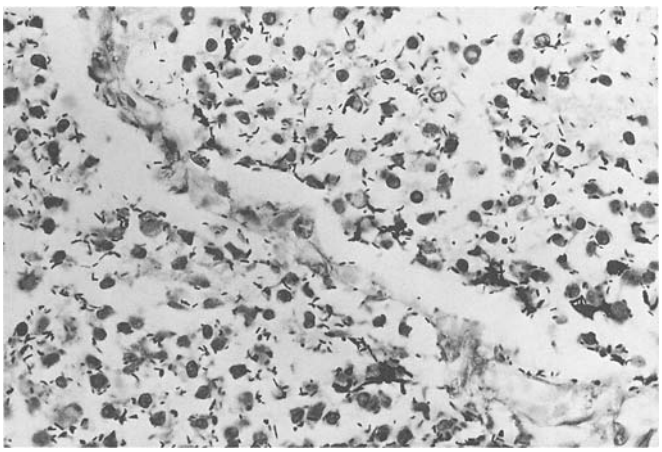

FIGURE 8.45. Pneumonic plague. Inflammatory cells and numerous argyrophilic coccobacilli of *Yersinia pestis* fill alveolar spaces. (Steiner silver impregnation method.)

Yersinia enterocolitica

Yersinia enterocolitica most frequently causes gastroenteritis and mesenteric adenitis, but may also cause septic arthritis[440] and overwhelming sepsis, particularly in patients with hemochromatosis.[441] Focal pneumonia is an uncommon manifestation of the infection.[442–444] Nodular infiltrates,[445] abscess formation,[446,447] and empyema[448] have also been described.

Miscellaneous Enteric Bacilli

A case of pneumonia has been attributed to Centers for Disease Control and Prevention (CDC) Enteric Group 15 currently classified as *Cedecea* species).[449]

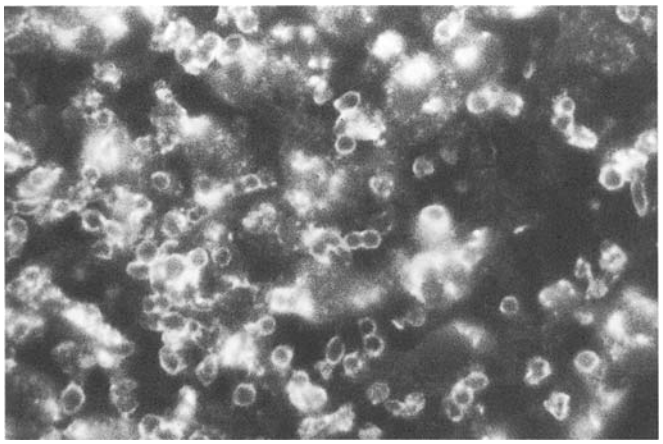

FIGURE 8.46. Compact intraalveolar aggregates of *Yersinia pestis* in formalin-fixed deparaffinized lung section are brightly fluorescent when stained with fluorescein-labeled immunoglobulins specific for this bacterium.

H. influenzae

H. influenzae was isolated with such high frequency from patients with influenza that early investigators attributed causality to the bacterium, not yet recognizing the existence of viruses.[450] The Pfeiffer bacillus was accepted by Opie and colleagues[126] as the cause of the underlying pneumonia when this group described the bacterial complications of influenza after World War I. Opie described the association of the gram-negative bacillus with purulent bronchitis in his patients, as opposed to the isolation of the gram-positive cocci more commonly from lung parenchyma. That association has been noted also by modern investigators, who were unable to distinguish clinically acute febrile tracheobronchitis from pneumonia except for the absence of radiographic infiltrates in the former.[451] MacCallum[125] also emphasized the bronchial association of the lesions in *H. influenzae* pneumonia, but challenged the etiologic association with the influenza syndrome. Some years later Pittman[452] defined the existence of strains that had polysaccharide capsules and others that were unencapsulated. Pittman noted the antigenic differences among the encapsulated isolates and first suggested that the type B strains were associated with severe bacteremic disease. The matter was finally settled when Smith and colleagues[453] isolated influenza virus by inoculation of ferrets in 1933. Meanwhile, deliberation as to the exact role of a preceding viral respiratory infection in the pathogenesis of *H. influenzae* pneumonia continues.[454]

MacCallum[125] noted a purulent exudate in the bronchi where the gram-negative bacilli could be demonstrated readily. The corresponding macroscopic appearance was of multiple nodular yellowish lesions and relatively intact lung parenchyma. In the air spaces, the infiltrate was either neutrophilic or mononuclear, and bacteria were difficult to demonstrate.

MacCallum described a fibrinous exudate frequently, but noted the rarity of empyema. This observation has been confirmed by others,[455] but empyema does occur in *H. influenzae* infection.[456,457] Likewise, suppuration and abscess formation are uncommon, but have been described.[456,458,459] Pneumatoceles similar to those found in staphylococcal pneumonia have also been described radiographically in children with *H. influenzae* infection.[460]

The pathogenesis of *Haemophilus* pneumonia has been reviewed by Moxon and Wilson.[461] Traditionally serious systemic infections were thought to be caused by encapsulated type B strains, usually in young children.[455,462,463] The first cases in adults were described by Keefer and Rammelkamp[464] in 1942, and this association has been emphasized increasingly in recent years.[465] In fact, adult infection by *H. influenzae* (primarily nontypeable strains) now constitutes approximately 15% of pneumonias.[466] Bacteremic pneumonia, which complicates roughly 12% of total *H. influenzae* pneumonia cases,[467] is usually caused by encapsulated type B strains,[468] but type C,[469] type D,[470] type E,[458] and type F strains[471] have also produced bacteremic disease and fatal infections. Type F encapsulated strains are the next most common after type B in bacteremic pneumonia.[468] Encapsulated *Haemophilus* produces a variety of diseases in adults that is similar to the spectrum of serious disease in children, including meningitis, arthritis, and epiglottitis as well as pneumonia.[472]

During the preceding decade, the use of polysaccharide-protein conjugate vaccines has become increasingly prevalent in developed nations.[467,473] The results, in the case of *H. influenzae* type B, have been astounding,[474] so much so that current medical students may never see a case of epiglottitis with a positive "thumb sign." Although cases of invasive non–type B disease continue, the numbers are not so large as to invite speculation that a new "niche" has been created for strains not targeted by vaccines.

The importance of nonencapsulated (nontypeable) strains has become recognized both in children[475–478] and in adults.[451,465] Nontypeable strains have been recorded as important pathogens in community- and hospital-acquired pneumonia in the elderly, accounting for 11% of episodes of pneumonia documented by transtracheal aspirates.[479] Nosocomial *Haemophilus* pneumonia has increasingly been recognized as a problem in younger patients also.[480] Bacteremia also occurs with nontypeable strains but less frequently than with type B *H. influenzae.*

Patients with *H. influenzae* pneumonia usually survive the acute episodes, so there has been very little recent description of the histopathology. Most reports emphasize the occurrence of both focal pneumonia (see Fig. 8.13) and lobar or segmental infiltrates, as defined by

chest radiographs.[451,458,465,479,481] It has been suggested that type F *H. influenzae* is particularly prone to cause lobar pneumonia.[482] Henry and colleagues[483] described an unusual case of *H. influenzae* pneumonia in which the clinical course was prolonged. Polymorphonuclear leukocytes, partial destruction of the bronchial walls, and fibroblastic proliferation were demonstrated in a lung biopsy.

The challenge of managing serious infections caused by *H. influenzae* has increased during the past three decades as bacterial resistance has developed to the two first-line chemotherapeutic agents, ampicillin and chloramphenicol. Decreased susceptibility to trimethoprim/sulfamethoxazole, to rifampin, and to some second-generation cephalosporins and β-lactam–β-lactamase inhibitor/combinations have also been documented, although their rate of isolation remains quite low.[484,485] The third-generation cephalosporin antibiotics now serve as the major therapeutic agents for serious systemic infection. The importance of interpretation of Gram stains of sputum by skilled observers has been emphasized by the frequency with which the thin, pleomorphic gram-negative *Haemophilus* bacteria in smears have been overlooked or misinterpreted by medical house officers and other observers.[458,465,486]

Other Haemophilus *Species*

Haemophilus parainfluenzae, a frequent component of the oropharyngeal flora, is not considered a pulmonary pathogen, but cases of pneumonia[487] and thoracic empyema have been described.[488] A single case of *Haemophilus aphrophilus* pneumonia in a previously normal child has been reported.[489] Pneumatoceles and a single abscess developed radiographically in the setting of diffuse air-space infiltrates.

Legionella *Species*

The newest addition to the list of major pulmonary pathogens is the genus *Legionella*.[490] As has been true of most recently discovered pathogens, the bacteria had been recognized for many years, but their true role in human disease had not been appreciated. It took the combined effects of a point-source outbreak and intense scrutiny from the media to generate the concerted investigations required to unravel the mystery.[111] After 15 years of study, there are now 41 validly published species within the genus, of which 17 have been reported to cause human disease. Several species contain multiple serotypes.[37] By far the most important human pathogen is *Legionella pneumophila*, which accounts for 75% or more of human infections.[491] Within the species *L. pneumophila*, serogroup 1 strains account for the majority of infections and serogroup 6 strains for most of the rest. The other pathogenic species of note is *Legionella micdadei*, named for Joseph McDade, who isolated the strains from the original epidemic of pneumonia at the American Legion convention in Philadelphia during the American bicentennial celebration.

Two clinically and epidemiologically distinct respiratory syndromes are caused by *Legionella* spp. The first, known as Pontiac fever, is an acute, self-limited flu-like syndrome that includes cough but not radiographic evidence of pulmonary infiltrates. The incubation period is very short, and the attack rate is very high. This syndrome has been caused by *L. pneumophila,*[492] *L. feeleii,*[493] *L. micdadei,*[494,495] and *L. anisa.*[496]

The second and most common manifestation of *Legionella* infection is acute pneumonia.[497] The original epidemic was known as legionnaires' disease, but the etiologic designation *Legionella* pneumonia is more generic. Much of the epidemiology and pathogenesis has been described in the introductory sections of this chapter, and the history of the disease has been reviewed.[111] The epidemic of mysterious respiratory disease at the Pennsylvania American Legion convention caused a great furor, because the country was ready for an outbreak of swine influenza, a strain with the antigenic characteristics of the swine virus having been isolated the previous year in Fort Dix, New Jersey. Influenza virus was not isolated until 1933,[453] so that strains from the great pandemic of 1918–1919 were not available, but serologic analysis had suggested that the epidemic was caused by a virus that resembled the swine strains isolated by Shope[498] the next year.

Epidemiologists pointed out that the great fall pandemic was preceded by a first wave of disease in the spring and summer. Epidemic pneumonia is unusual in the summertime, and the sudden appearance of a mysterious epidemic caught the attention of the media and put great pressure on the epidemic investigators. Initial investigations of the tissues from Philadelphia failed to identify an etiologic agent, and speculations about chemical intoxication began to emerge. During the pathologic analysis bacteria were seen in the tissues, but they were dismissed as secondary invaders. An expert panel was convened to review the pathologic evidence, which consisted of tissue blocks and sections retrieved from the hospitals where convention attendees had died. After reviewing the macroscopic pathology from the first Burlington epidemic of *Legionella* pneumonia the next year, one of the members of this panel commented that he thought the panel would not have been sidetracked if it had had the benefit of seeing the whole lungs (Charles Carrington, personal communication).

The experience with legionnaires' disease had a major impact on microbiologists and infectious disease clinicians, who were beginning to realize emphatically that the age of infectious diseases was still with us. A few years later astute investigators in Charlottesville, Virginia,[499]

and Pittsburgh, Pennsylvania,[500] recognized an unusual combination of events: acute purulent pneumonia in immunosuppressed patients, the presence of acid-fast bacilli in sections, and cultures negative for bacteria and mycobacteria. Using techniques that had been employed in the investigation of the American Legion epidemic, the Pittsburgh investigators isolated a gram-negative bacillus that was originally called the Pittsburgh Pneumonia Agent and later classified as *Legionella micdadei.* This species has the unusual characteristic of partial acid-fastness in tissues and secretions but not after growth on agar. The bacterium also undergoes morphologic transformation ultrastructurally, which may correlate with the changes in acid-fastness.[501] Surgical pathologists can learn a lesson from this experience: When the population of patients is sufficiently immunosuppressed and *either* clinical or pathologic markers of infection are present, use of a battery of special stains should be encouraged. The rules that define which organism should be sought as an etiologic agent for a certain type of inflammation must be thrown out the window. Appropriate special stains are discussed in the section on Morphologic Detection and Identification of Bacteria, above.

Most of our information about the pathology of *Legionella* infections has come from study of *Legionella micdadei* and serogroups 1 and 6 *L. pneumophila* infections. The pathologic information on these recently recognized pathogens is, in fact, far more complete than the data we have on such venerable bacteria as *Streptococcus, Staphylococcus, Haemophilus*, and enteric gram-negative bacilli.

Most of the pathologic features of *L. pneumophila* pneumonia were defined during the study of the 1976 epidemic in Philadelphia[502] and the 1977 epidemics in Los Angeles[503] and in Burlington, Vermont.[208,210] The review of the pathology of *Legionella* pneumonia by Winn and Myerowitz[211] remains current. The macroscopic distribution of lesions is most frequently focal and lobular, as has been illustrated in Fig. 8.3.[208] Multilobar involvement is common, however, and extensive confluence of the focal consolidation may produce the familiar difficulty in deciding whether extensive lesions are lobar or confluent lobular in nature. Cases of lobar pneumonia caused by *Legionella* spp. have been described by several investigators.[211,502–504] A careful study of serial macroscopic sections and paper-mounted whole-lung sections suggested that most cases were best described as confluent lobular pneumonia.[208] It should be noted that the infiltrates from patients in that same epidemic were frequently described as lobar radiographically.[209]

A prominent subset of *Legionella* pneumonias present as poorly marginated rounded opacities that may suggest neoplastic disease (see Figs. 8.14 and 8.15). Localized, poorly marginated opacities have been described in chest radiographs of both patients with *Legionella pneumophila*[505–507] and those with *Legionella micdadei*[508] pneumonia. Winn and Myerowitz[211] noted that distinct round lesions were present in 12 of 42 cases that could be analyzed. The macroscopic lesions have a tan-white appearance and a very friable texture because of the high fibrin content.

Initial reports did not emphasize abscess formation, but subsequent studies documented the potential for destructive pneumonia.[219,220,509–513] In a large series macroscopic abscesses were documented in 10 of 42 cases of *L. pneumophila* pneumonia and in five of nine cases caused by *Legionella micdadei.*[211] Most abscesses are small and are not demonstrable radiographically. The presence of cavitary lesions, however, is entirely compatible with *Legionella* pneumonia.[505,514] Small pleural effusions are common in *Legionella* pneumonia. Large effusions and empyema have been reported only rarely in cases caused by *L. pneumophila,*[5211,506,515] *L. micdadei,*[516] and *L. bozemanii.*[517,518]

Microscopically, the inflammatory infiltrate in *Legionella* pneumonia is variable. In approximately one third of cases the infiltrate is composed predominantly of polymorphonuclear neutrophils (Fig. 8.47); in one third, monocytes/macrophages predominate (Fig. 8.48); in the final third there is a mixture of macrophages and neutrophilic leukocytes. Fibrin is a prominent part of the exudate (Fig. 8.49), and hemorrhage in the air spaces is common. Edema and a sparse cellular infiltrate are seen around the periphery of active lesions. The interstitium is also frequently cellular but always considerably less so than the adjacent air spaces.

One of the distinctive features of *Legionella* pneumonia is an intense lytic process in the inflammatory exudate, leaving many nuclear fragments and a dusty appearance, dubbed leukocytoclastic by analogy to the noninfectious

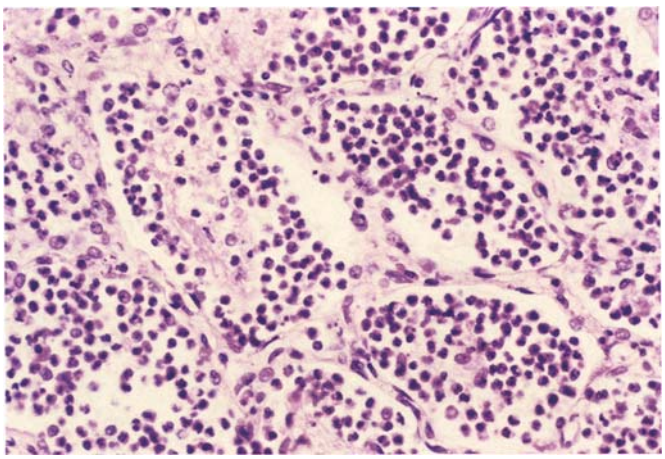

FIGURE 8.47. *Legionella pneumophila* pneumonia. Air spaces are infiltrated with intact polymorphonuclear leukocytes. *Legionella pneumophila* serogroup 1 is demonstrated in lung tissue by direct immunofluorescence.

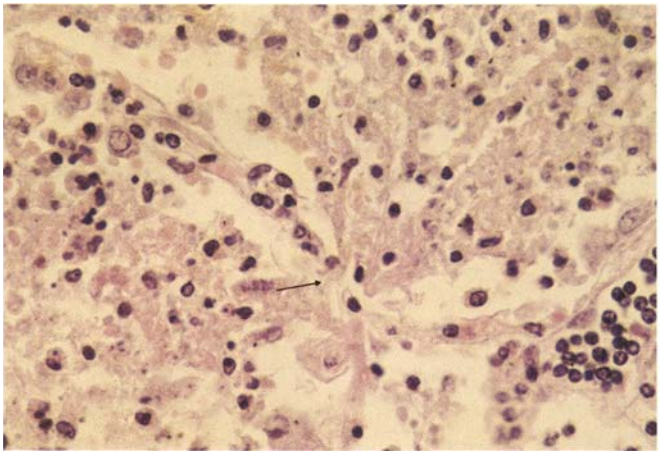

FIGURE 8.48. *Legionella pneumophila* pneumonia. Air space is filled with macrophages, some of which are undergoing disintegration. Note extension of the inflammatory exudate from one air space to another through the pore of Kohn.

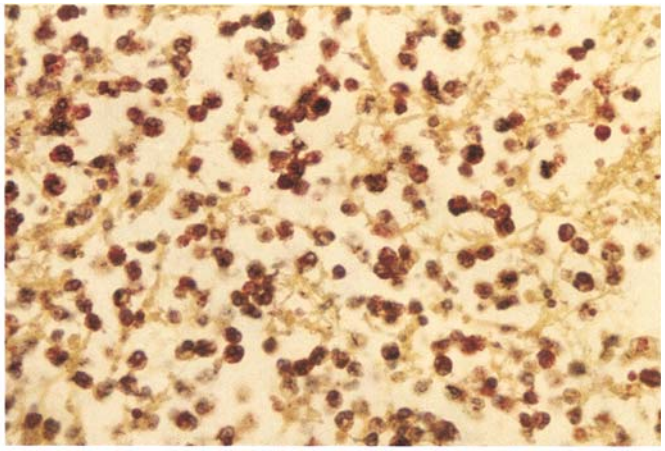

FIGURE 8.50. *Legionella pneumophila* pneumonia. Leder stain colors the granules of polymorphonuclear neutrophils and highlights these cells in inflammatory exudate. *Legionella pneumophila* is isolated in pure culture from a postmortem lung. (Leder procedure.)

cutaneous vasculitis (Fig. 8.49). In its extreme form the lysis of the infiltrate may be so complete that the H&E-stained exudate has a bluish homogeneous appearance. Weisenburger and colleagues[519] have described an acellular fibrinoserous exudate in the air spaces of neutropenic patients. Some of the confusion about the nature of the cellular exudate in *Legionella* infection derives from the difficulty of distinguishing cell types when the exudate is undergoing lysis. In such cases application of the Leder stain[520] for neutrophils may resolve the issue (Fig. 8.50).

Diffuse alveolar damage, including hyaline membranes remote from the primary inflammatory foci, were found in 13 of 53 cases of *L. pneumophila* pneumonia and in one of seven autopsied cases of *L. micdadei* pneumonia.[211] An expected etiology for diffuse alveolar damage was usually present, but some cases could not he explained by factors other than *Legionella*, and a lung biopsy was reported in which diffuse alveolar damage was the only pathologic finding.[225]

Coagulative necrosis, which is present in a minority of cases and may mimic pulmonary infarcts, may be associated with vasculitis.[211,503] Small pulmonary vessels are infiltrated with inflammatory cells and often contain thrombi (Fig. 8.51). In contrast to *Pseudomonas* vasculitis, bacteria are infrequent in the damaged blood vessels. Vasculitis may occur without coagulative necrosis, however. In one series vasculitis was seen in 16 of 53 cases

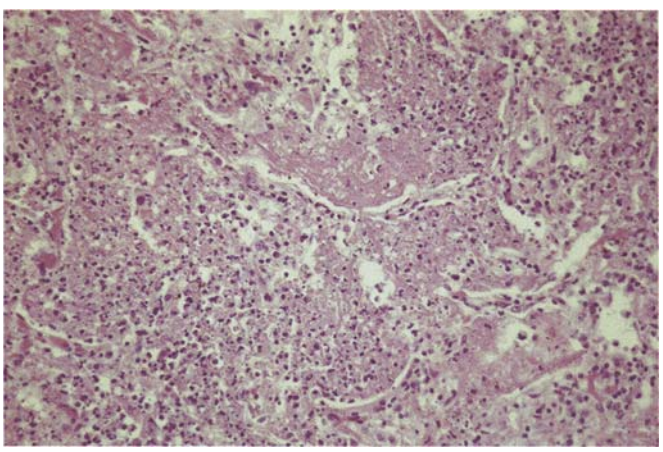

FIGURE 8.49. *Legionella pneumophila* pneumonia. Air spaces are filled with fibrin and a cellular exudate, which is undergoing extensive lysis, leaving karyorrhectic debris.

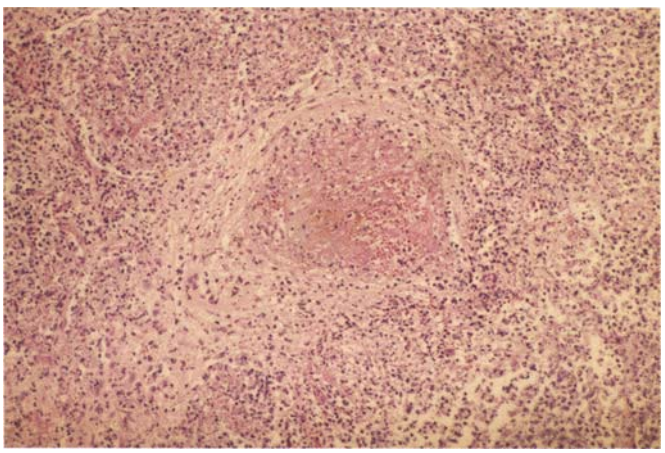

FIGURE 8.51. *Legionella pneumophila* pneumonia. Necrosis of pulmonary blood vessel, possibly pulmonary vein, with inflammatory cell infiltration of wall. *Legionella pneumophila* is isolated in pure culture from a postmortem lung.

of *L. pneumophila* pneumonia (30%), but coagulative necrosis was found in only six cases, all of which contained inflamed blood vessels.[211]

Ultrastructural examination of tissues has expanded our knowledge of the cellular interactions with *Legionella* and provided some useful pathogenetic clues.[501,521] The initial ultrastructural examinations documented the gram-negative cell wall structure of the bacteria and the close association of the bacteria with macrophages in the air-space infiltrate (Fig. 8.52). Most of the specimens were obtained at autopsy, however, and cells were insufficiently preserved to make definitive observations of subcellular structure. Glavin and colleagues[522] examined three lung biopsies that had been freshly fixed in glutaraldehyde solution. They noted the association of phagocytosed *Legionella* with alveolar macrophages (Fig. 8.53) but also described phagocytosed organisms in polymorphonuclear neutrophils. They further noted that the intracellular bacteria were in vacuoles that had closely apposed, ribosome-like structures (Fig. 8.54). This association of bacteria with ribosome-studded phagosomes has been noted experimentally also.[523] The association appears related to intracellular parasitism, but the precise function associated with the physical relationship is not known.[524]

Legionella spp. can be demonstrated in tissue readily, a considerable irony because the failure to visualize them delayed the recognition of the cause of the Philadelphia outbreak. As usual, the task is easy once the ground has been broken. A monoclonal fluorescent reagent that reacts with all serogroups of *L. pneumophila* is commercially available. This reagent has the advantage of good specificity, but does not detect other species.[525,526] Even the monoclonal reagent, however, may cross-react with other bacteria, such as spores of *Bacillus cereus*[527] and *Bordetella pertussis*.[528] Polyclonal fluorescent reagents that react with many species have also been evaluated and are commercially available,[525,529] but it is important to remember that cross-reactions with other bacterial species will occur.

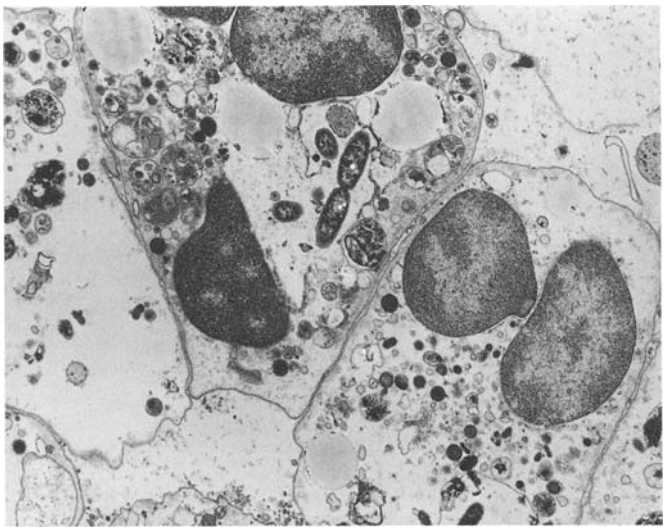

FIGURE 8.53. *Legionella pneumophila* serogroup 1 pneumonia. Bacteria are phagocytosed by inflammatory cells, probably polymorphonuclear neutrophils, that are undergoing extensive degeneration. One bacterium is undergoing binary fission, suggesting intracellular multiplication. (Glutaraldehyde-fixed, osmicated lung biopsy, uranyl acetate stain.)

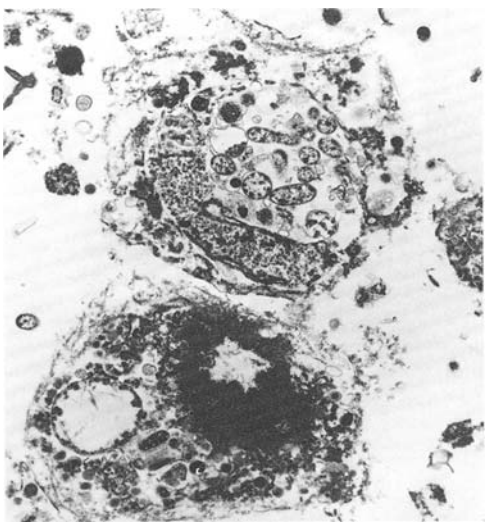

FIGURE 8.52. *Legionella pneumophila* serogroup 1 pneumonia. Multiple bacteria have been engulfed by or multiplied in an alveolar macrophage in the air space. Phagocytosed debris and bacteria are present in the adjacent macrophage. (Formalin-fixed lung tissue with uranyl acetate stain.)

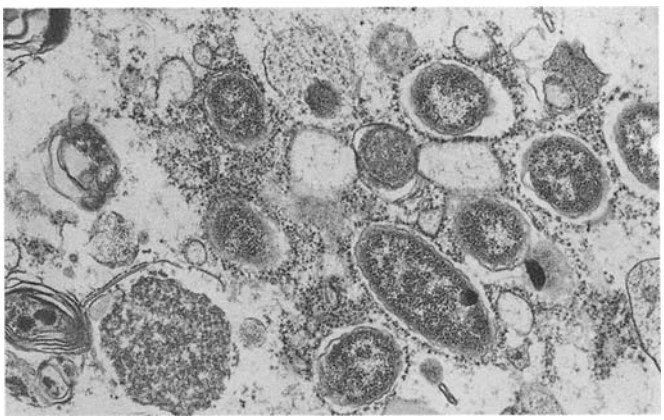

FIGURE 8.54. *Legionella pneumophila* serogroup 1 pneumonia. Bacteria have been taken up by macrophage into phagosomes that are lined by ribosome-like structures. (Glutaraldehyde-fixed lung biopsy, uranyl acetate stain. Courtesy of Dr. Frederick Glavin.)

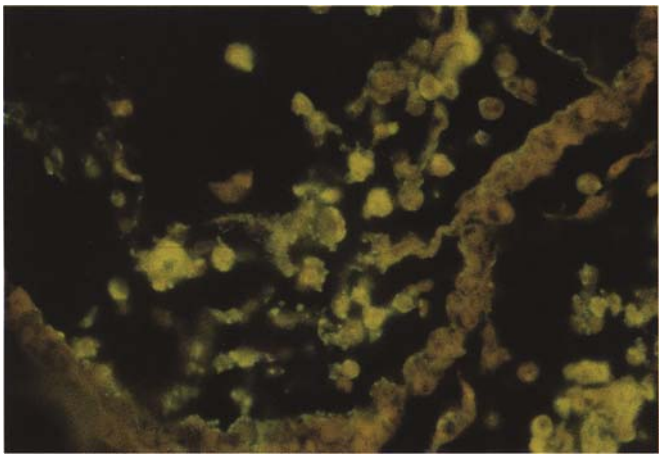

FIGURE 8.55. *Legionella pneumophila* pneumonia. Phagocytic cells in air spaces contain fluorescent bacteria and antigenic debris. Formalin-fixed, paraffin-embedded lung tissue has been reacted with fluorescein-conjugated antiserum to *Legionella pneumophila.*

Legionella antigen survives prolonged formalin fixation and processing for paraffin-embedded sections, so that special manipulations are not needed to visualize the bacteria in archival material (Figs. 8.55 and 8.56). If formalin-fixed tissue is available, a much simpler method for preparation of smears is to scrape the surface of fibrinous lesions with a scalpel blade and transfer the exudate to clean glass slides.

The traditional adaptations of Gram stain for tissue, such as the Brown–Hopps and Brown and Brenn versions, do not color *Legionella* and some other fastidious gram-negative bacilli well. Unfortunately, our experience is that considerable variation still occurs from laboratory to laboratory.

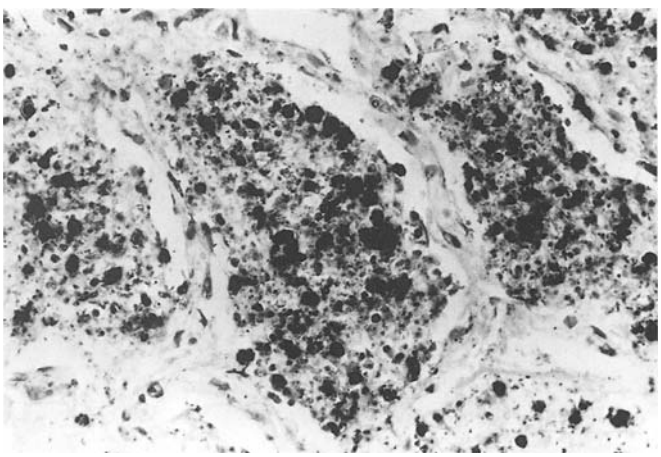

FIGURE 8.56. *Legionella pneumophila* pneumonia. Air spaces are filled with inflammatory exudate that is heavily stained by antibody to *Legionella pneumophila,* which has been conjugated to immunoperoxidase.

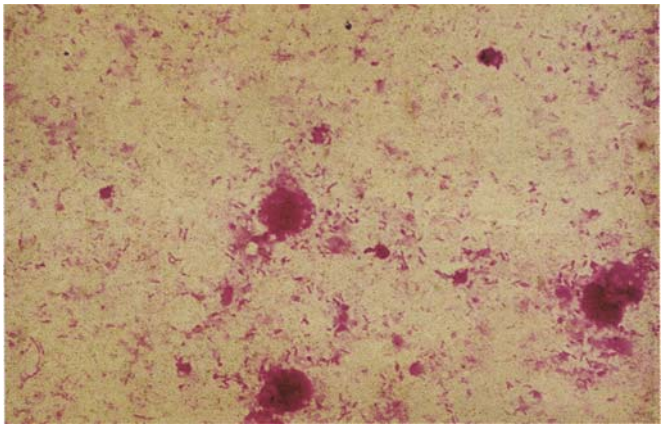

FIGURE 8.57. *Legionella pneumophila* pneumonia. Gram stain of lung imprint shows multiple thin, somewhat pleomorphic gram-negative bacilli. *Legionella pneumophila* serogroup 1 is isolated in pure culture from the lung.

With some forethought the problem may be avoided, because scrapings from the surface of the lung or impression smears may be prepared and stained with the traditional Gram stain (Fig. 8.57). The staining of fastidious gram-negative bacilli can be enhanced greatly by addition of basic fuchsin (0.5 g/L) to the safranin counterstain. The successful demonstration of *L. pneumophila* in tissue was first accomplished reliably by use of the Dieterle silver impregnation stain, first intended for demonstration of spirochetes.[530,531] Other silver stains, such as the Steiner (Fig. 8.58)[532] or Warthin–Starry[533] modifications, also stain the bacteria well. The silver-stained

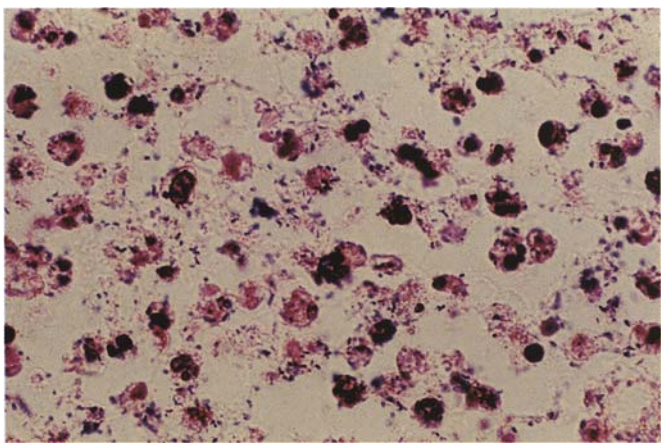

FIGURE 8.58. *Legionella pneumophila* pneumonia. Innumerable bacilli are demonstrated by silver impregnation stain of airspace exudate. Many bacteria are cell associated. Diffuse bacterial antigen revealed by immunologic procedures is not revealed by this stain. (× 250.) Fine morphology discernible with Gram stain is obscured by heavy deposition of silver salts. (Steiner silver impregnation method.)

bacteria appear larger and more regular than do Gram-stained organisms because of the deposition of silver on the surface of the bacteria. The information on Gram reactivity is lost when the silver impregnation stains are employed. Other stains such as the Giemsa[534] or Gimenez[535] procedures may also be used but offer little additional advantage. All of these chemical stains are immunologically nonspecific. They have the advantage of not being limited by serologic specificity, but they provide little or no information as to the nature of the bacterium. The temptation to ascribe a genus and species designation to bacteria in tissue should be resolutely resisted.

The radiographic abnormalities in *Legionella* pneumonia resolve slowly, and patients may have a prolonged convalescence.[514,536] Focal organization of infiltrate has been noted occasionally in fatal acute cases,[503] not surprising in view of the accompanying necrosis. A few cases of chronic organizing pneumonia have been attributed to *Legionella.*[537-539] In most of these cases the diagnosis was made serologically and the patients experienced a prolonged, complicated clinical course. Chastre and colleagues[540] have described five well-documented cases of pulmonary fibrosis following acute *L. pneumophila* infection. Two patterns of fibrosis were observed, one in which the interstitium was primarily involved, and a second in which the air spaces were the principal site of fibrosis. In both patterns there was ultrastructural damage to the alveolar epithelial lining and basement membrane. Types 1 and 3 collagen were demonstrated in the areas of interstitial and intraalveolar fibrosis.

Walsh and Kelley[226] described a patient with acute pneumonia and a plasma cell infiltrate in the lung. Serogroup 1 *L. pneumophila* was isolated in pure culture from the lung of this patient, but the significance of the unusual infiltrate is hard to assess.

Control of *Legionella* pneumonia consists of preventive measures to eradicate environmental bacteria and antimicrobial chemotherapy of patients using erythromycin. Some antibiotics that are effective against *Legionella in* vitro, such as aminoglycosides, are not active in vivo, probably because the antimicrobial agents do not accumulate intracellularly in macrophages.[541]

Other Legionella *spp.*

Many of the other defined species of *Legionella* have been isolated from cases of pneumonia.[37] The limited information from the few cases of each species described suggest that the pathologic response to infection is similar to that found in *L. pneumophila* and *L. micdadei* infections.[211] Some of the recently characterized strains have only been isolated from the environment, but from past experience it can be expected that any species may cause human disease in a sufficiently immunosuppressed patient.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is the most virulent species of the genus and the predominant isolate in clinical microbiology laboratories. In contrast to the Enterobacteriaceae, this species of *Pseudomonas* produces indophenol oxidase, providing an easy differentiation for the microbiologist. *Pseudomonas* spp. are gram-negative bacilli, but tend to be thin and long compared to the shorter, fatter enteric bacilli. *P. aeruginosa* produces powerful exoenzymes, such as proteases and elastases, that contribute to tissue destruction. Consequently the hallmarks of *Pseudomonas* infections are hemorrhage, necrosis, and abscess formation.

There are two pathogenic mechanisms for *P. aeruginosa* pneumonia. The first is aspiration of oral contents, usually in a nosocomial setting after shifting of the oral flora to gram-negative species.[32] Patients who are at risk usually have a serious underlying disease that is immunosuppressive, particularly acute leukemia.[138,218,304,542-545] Bodey and colleagues[544] reported that *Pseudomonas* bacteremia was 19 times more frequent in patients with acute leukemia than in those with solid tumors. In recent years AIDS has joined the list of predisposing conditions. Patients with HIV infection may develop *P. aeruginosa* pneumonia as a nosocomial infection, but traditional causes of community-acquired bacterial pneumonia appear to be more common etiologic agents.[142,546] Neutropenia is a particularly important risk factor. In their series of patients with *P. aeruginosa* bacteremia, Bodey and colleagues noted that 69% of patients had absolute initial neutrophil counts less than 1000/mm^3 and 46% had initial neutrophil counts less than 100/mm^3. Recovery from the infectious episode is often as dependent on recovery of neutrophils as on antimicrobial therapy.[138]

The Infectious Diseases Society of America has issued guidelines for the antimicrobial chemoprophylaxis of febrile neutropenic patients, each of which includes an antipseudomonal agent.[547] Maschmeyer and Braveny[548] point out, however, that while some individual studies document a decline in the incidence of *Pseudomonas* infection among this patient population, an overall clinical benefit of such prophylactic therapy is not yet proved. Furthermore, the authors suggest, other relevant factors that contribute to mortality must be considered when compiling and analyzing such data.

In some series chronic cardiopulmonary disease was a common underlying condition,[549,550] but in other series these diseases have not been represented,[138,545] perhaps because of the nature of the patient populations served. Bacteremic infection appears to be more common when the patient populations are severely immunosuppressed or leukopenic.

Bacteremia is common in *Pseudomonas* pneumonia. Unger and colleagues[403] noted positive blood cultures in

eight of 19 cases, in all of which the pneumonia was the initial event. Metastatic infection, such as vertebral osteomyelitis[551] and ecthyma gangrenosum, are frequent complications of the bacteremia.[552] The mortality in patients who are bacteremic is very high.

Pseudomonas aeruginosa pneumonia is rarely acquired in the community and in the absence of immunosuppression, but has been described in patients with status asthmaticus,[553] hot-tub associated pneumonia,[554] and in previously healthy individuals.[552,555–557] Rare fatal instances have also been reported.[558]

The second pathogenetic mechanism for *P. aeruginosa* pneumonia is secondary spread to the lungs from distant foci through the bloodstream (Fig. 8.59).[559–561] The early radiographic appearance of the lungs may suggest congestion[545] or even unilateral pulmonary edema,[562] but by the time the patient dies necrotizing or hemorrhagic inflammatory lesions predominate.

A third, alternative pathogenic mechanism of infection, pertaining primarily to intubated patients, has also been proposed.[563–565] Investigators found that *Pseudomonas* infection of the tracheobronchial tree sometimes precedes the development of oropharyngeal colonization, as determined by site-specific culture techniques and corroborated by molecular epidemiologic techniques. The implication, therefore, is that direct inoculation into the lower respiratory tract, probably during mechanical suctioning, may set up the development of pneumonia in mechanically ventilated patients without prior oropharyngeal colonization.

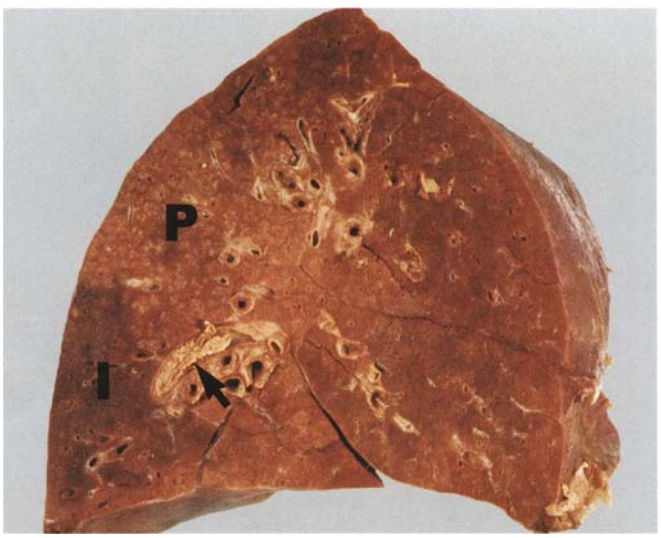

FIGURE 8.59. *Pseudomonas aeruginosa* pneumonia. Multiple yellowish-white nodules represent focal pneumonia (P). In an adjacent area of the lung there is a thromboembolus (arrow) and a pleural-based peripheral focus of hemorrhagic, septic infarction.

The pathologic presentation of *Pseudomonas* pneumonia may be varied even in an individual patient. There may be focal nodular lesions, abscesses, and/or hemorrhage. Thromboemboli may be accompanied by pleural-based hemorrhagic septic infarcts (Fig. 8.59).

Tillotson and Lerner[549] described the pathology of eight fatal cases of *P. aeruginosa* pneumonia at autopsy. Criteria for inclusion in the study were either (1) isolation of the bacterium from two or more successive sputa, (2) isolation of the bacterium from one sputum and blood, or (3) isolation of the bacterium from pleural fluid. It is likely that most of these cases represented primary *Pseudomonas* pneumonia. Macroscopically there were extensive confluent focal pneumonia and pleural adhesions. There was a variable histopathologic picture within each case. Some areas that contained intense inflammatory exudates were associated with microabscesses and destruction of alveolar septa (Fig. 8.60). In other sites the lesions were hemorrhagic, but deficient in leukocytes, while still other sites contained edema and a moderate mixed inflammatory exudate in the air spaces. Thrombosis and necrosis of vascular walls were not observed.

Rose and colleagues,[550] who used diagnostic criteria similar to those employed by Tillotson and Lerner, described air-space infiltrates as the predominant radiographic appearance and a polymorphonuclear neutrophilic exudate as the microscopic appearance of all 19 patients in their study. Necrosis of alveolar septa occurred in 16 patients, and 14 patients had abscesses that exceeded 1.0 cm in diameter.

Fetzer and colleagues[218] described two types of macroscopic pathology in *Pseudomonas* pneumonia in seven patients. Criteria for inclusion in the study were (1) a positive antemortem culture from blood, (2) a positive postmortem culture from lung, and (3) a postmortem diagnosis of pneumonia. Extrapulmonary inflammatory lesions were described, but the number of patients affected was not stated.

The first macroscopic pattern was characterized by focal nodular, poorly delimited, hemorrhagic lesions that were often located in a subpleural location and resembled noninfectious pulmonary hemorrhages. Microscopically these lesions were hemorrhagic, noninflammatory, and contained many gram-negative bacteria. The lesions were centered around small pulmonary blood vessels. Necrosis of alveolar septa was variably present.

The second macroscopic pattern was characterized by firm, yellow-brown or tan necrotic nodules that were elevated above the cut surface and sharply delimited from the surrounding lung tissue.[218] Microscopically these lesions had two slightly different appearances. In one subset there was a mixed inflammatory infiltrate that consisted of lymphocytes, macrophages, and polymorphonuclear

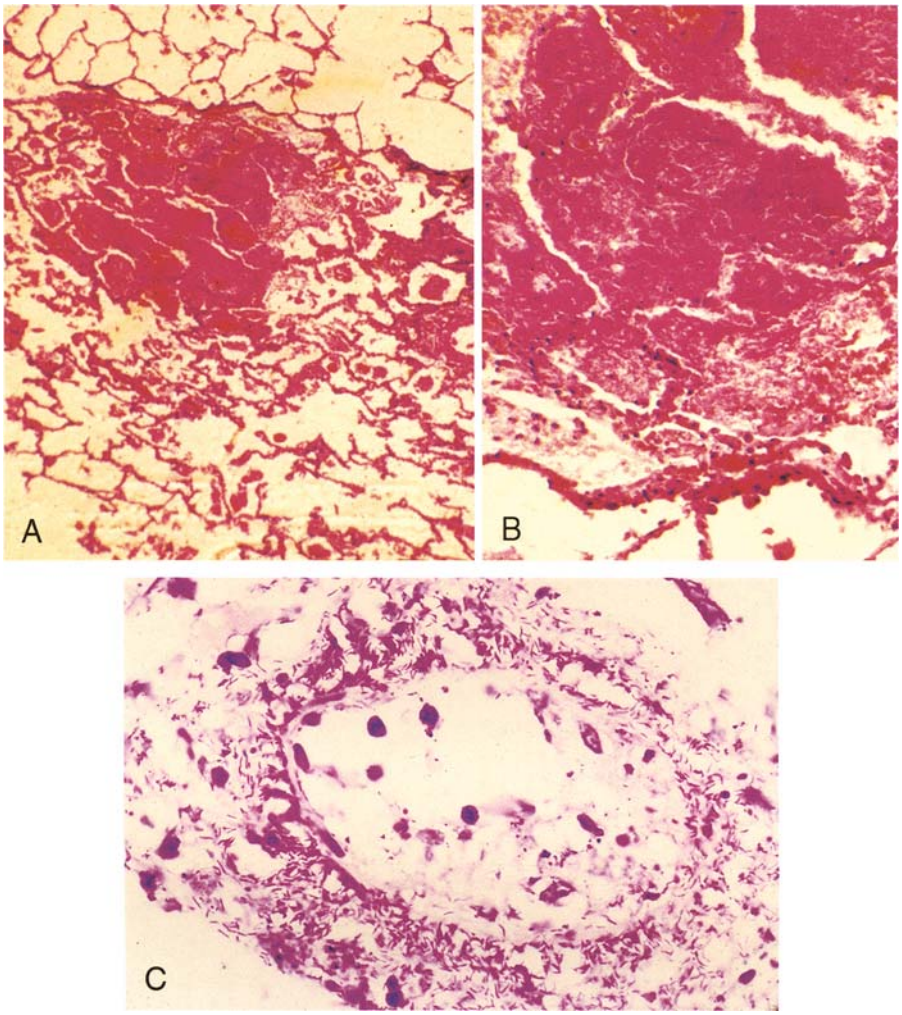

FIGURE 8.60. *Pseudomonas aeruginosa* pneumonia in a patient with *Pseudomonas* sepsis. **A.** Multiple foci of inflammation reflect the bacteremic spread to the lungs. **B.** Intense neutrophilic inflammation has produced a microabscess with destruction of alveolar septa. *Pseudomonas aeruginosa* was isolated from blood antemortem and from the lung in pure culture. **C.** *Pseudomonas vasculitis*. The blood vessel is impregnated with gram-negative rods (Brown-Brenn).

neutrophils that had undergone extensive lysis. Liquefactive necrosis and abscess formation were common. Bacteria, which were sparse, lined the walls of septal capillaries at the periphery of the lesion. Thrombosis was uncommon and bacterial invasion of muscular arteries was minimal.

The more common histopathologic pattern in these yellow, necrotic nodules was distinctive and suggested a diagnosis of *P. aeruginosa* infection. This pattern was characterized by vasculitis and coagulative necrosis. Small muscular arteries and veins were necrotic, hyalinized, and infiltrated with leukocytes, which often lined the endothelial surface. Thrombi were uncommonly present. Large numbers of gram-negative bacilli, however, were present in the blood vessels, concentrated on the adventitial surfaces. The bacterial aggregates were never as prominent as those seen in other organs. Coagulative necrosis was accompanied by large numbers of bacteria and few inflammatory cells.

The vasculitic lesions may resemble pulmonary infarcts. Soave and colleagues[560] described a woman with rheumatic heart disease and systemic lupus erythematosus who developed a rapidly progressing pneumonia accompanied by bacteremia and metastatic necrotic lesions in the skin and other organs. Scant *P. aeruginosa* had been isolated from the sputum 2 days earlier. An extrapulmonary source for the infection was not evident. At autopsy there was patchy consolidation throughout the lungs, and numerous 2- to 4-mm, gray-yellow nodules were present. Microscopically there was extensive focal pneumonia with microabscess formation and diffuse hemorrhagic necrosis. Vascular necrosis and infiltration by bacteria occurred, but intraluminal organisms were not seen. Similar lesions were present in other organs.

Swartz and Castleman[559] discussed a patient in the case records of the Massachusetts General Hospital who was similar to the patient reported by Soave, except that extensive burn wounds with suppurative infection were present. Septic infarcts of the lung and focal pneumonia developed in close temporal proximity to *Pseudomonas* bacteremia, and multifocal abscesses were present in the kidneys. At autopsy the pulmonary vasculature was focally necrotic, and bacteria were seen in the walls of the blood vessels.

The pulmonary vasculitis has been reproduced by Teplitz.[566] He produced a fatal wound sepsis in rats by inducing an extensive full-thickness burn of the skin followed by infection with clinical strains of *P. aeruginosa.* Hemorrhagic subpleural pulmonary lesions resulted. Of the animals that developed metastatic infection, 30% also developed vascular lesions that were very similar to the vasculitis in humans. Although the bacteria had clearly reached the lungs through the bloodstream, large numbers of bacilli were densely packed in the medial and adventitial layers or concentrated as partial or circumferential perivascular bacterial cuffs without appreciable invasion of the media. Bonifacio and colleagues[567] substantiated these findings in an autopsy series of eight infants who died of *Pseudomonas* pneumonia. Inclusion criteria were the antemortem isolation of *P. aeruginosa* from the blood or trachea and age less than 1 year. Once again the two major histopathologic patterns described were "a paucicellular coagulative confluent bronchopneumonia with perivascular bacillary infiltration" and "a more usual cellular 'abscess-forming' bronchopneumonia without perivascular infiltration by bacteria." All cases were associated with some degree of alveolar hemorrhage.

In summary, the most common macroscopic manifestations of *Pseudomonas* pneumonia are confluent focal pneumonia with abscess formation, focal pulmonary hemorrhage, focal nodular necrotic lesions, or septic infarcts. Microscopically, a similar spectrum included necrotizing acute inflammation, relatively noninflammatory hemorrhage into the air spaces with focal acellular necrosis, and coagulation necrosis with necrotic pulmonary muscular veins and arteries. It is difficult to distinguish between aspiration and bacteremic pathogenetic mechanisms in the reports of human disease. Several lines of evidence suggest, however, that the vasculitis and septic infarcts occur predominantly in pneumonia that is secondary to bacteremia. Although the bacteria are concentrated in the external layers of the blood vessels, identical lesions can be produced experimentally when bacteremia originates in a septic burn wound. The same sequence of events has been described in humans. The concentration of the pulmonary lesions around small arteries and veins, rather than around terminal and respiratory bronchioles, also suggests a vascular focus. It appears that this very characteristic *Pseudomonas* vasculitis may be decreasing in frequency as the antimicrobial therapy for *P. aeruginosa* has become more effective.

The course of *Pseudomonas* pneumonia is acute, but a protracted clinical course has been described in occasional cases.[550] Patients with cystic fibrosis are commonly colonized with *P. aeruginosa* late in the course of their disease, often after initial colonization with *S. aureus* or *H. influenzae.*[297,568] The characteristic pulmonary lesion in cystic fibrosis is bronchiectasis. The colonizing bacteria often produce chronic infection with periodic acute exacerbations of symptoms, although the initiation of a colonized state generally heralds a gradual decline in pulmonary function (see Chapter 5).[569] Recovery from infection (or "clearance") is thought to be hampered by a variety of host and pathogen factors: (1) impaired mucociliary clearance of luminal secretions and organisms, (2) decreased production of antimicrobial peptides (defensins) in alveoli, (3) decreased production of nitric oxide, and (4) the formation of biofilms by the organism.[568,570] A close association has been documented between very mucoid strains of *P. aeruginosa* and cystic fibrosis.[297,568] Rivera and Nicotra[571] suggested that the association may be with bronchiectasis rather than with cystic fibrosis per se. The presence of a mononuclear infiltrate in some lesions of *P. aeruginosa* pneumonia has been noted. Tillotson and Lerner[549] also emphasized the coexistence of predominantly mononuclear infiltrates with liquefactive neutrophilic lesions.

The sputum contains *P. aeruginosa* in approximately 80% of cases, but as it often also contains other pathogens careful clinical correlation or additional diagnostic studies must be performed. In various reports, 20% to 50% of patients with primary pneumonia had an accompanying bacteremia.[138,549,550]

Other Pseudomonas *Species and Non–Glucose-Fermenting Bacteria*

Burkholderia cepacia

Burkholderia cepacia (formerly *Pseudomonas cepacia*) has recently been recognized as an important pathogen in patients with cystic fibrosis (CF). As occurs with *P. aeruginosa*, infection is associated with a noticeable worsening of the clinical condition, although an acutely fatal form of disease, termed the cepacia syndrome, has become widely recognized.[572,573] Reliable recovery of this bacterium from sputum may require selective media to inhibit overgrowth by *P. aeruginosa.* This species is usually resistant to aminoglycoside antibiotics. The inherent resistance of *B. cepacia* to antimicrobial agents, its propensity for transmission from person to person (occasionally leading to epidemics among patients who have cystic fibrosis),[572] the severity of clinical manifestations, and the proposals that the organism be used as an agricultural pesticide highlight the prominence of this formerly

exotic bacterium as a problem for patients with cystic fibrosis.[573]

Tomashefski and colleagues[574] have described the pathologic patterns of *B. cepacia* pneumonia at autopsy in 40 patients. Lobular or peribronchial pneumonia was the most common pathologic manifestation of infection. Well-delineated yellow peribronchial nodules were present (Fig. 8.61). Diffuse alveolar damage was uncommon, and vasculitis was not observed. The first group of patients had a rapidly progressing clinical course; purulent focal pneumonia occurred most frequently in this group. Polymorphonuclear neutrophils were prominent and abscesses were a regular feature (Fig. 8.62). The second group experienced slow clinical deterioration after colonization with *B. cepacia.* Chronic inflammatory lesions, including macrophages, lymphocytes, and plasma cells, were more commonly found in this group. Chronic interstitial pneumonia with or without an organizing alveolar component was found almost exclusively in this group. Lesions of varying types occurred in each clinical category, however.

In Toronto, Ontario, where the frequency with which patients in CF clinics are colonized with *B. cepacia* may approach 40%, investigators have conducted immunolocalization studies of explanted lung tissue from CF patients with a variety of clinical scenarios.[575] Although no correlations could be established among bacterial load and distribution, degree of pathology, and outcome of lung transplantation, the more destructive histopathologic lesions, including necrosis and abscess formation, were more prominent in those patients succumbing to cepacia syndrome. Also, whereas previous reports have demonstrated *P. aeruginosa* localization primarily within the airways,[576,577] Sajjan and coworkers[575] found *B cepacia* distributed widely throughout the lungs, including between and beneath epithelial cells, and within alveolar walls and alveolar macrophages.

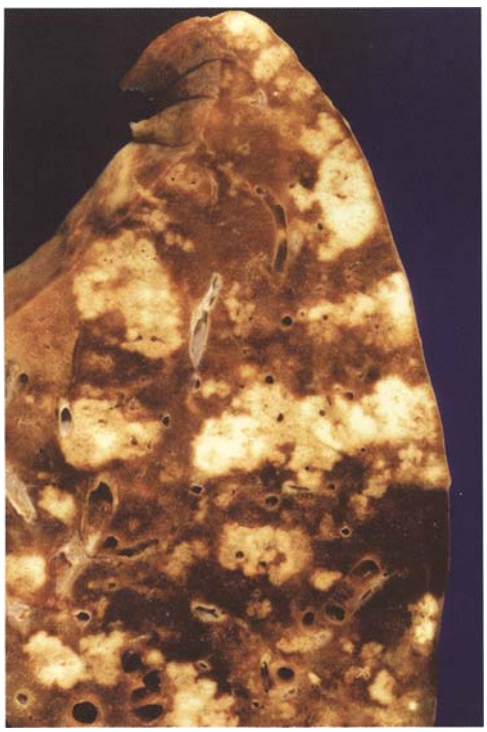

FIGURE 8.61. *Burkholderia cepacia* pneumonia. Multiple yellow consolidative nodules are centered on the bronchi and bronchioles. (Courtesy of Dr. Joseph F. Tomashefski, Jr., MetroHealth Medical Center, Cleveland, OH.)

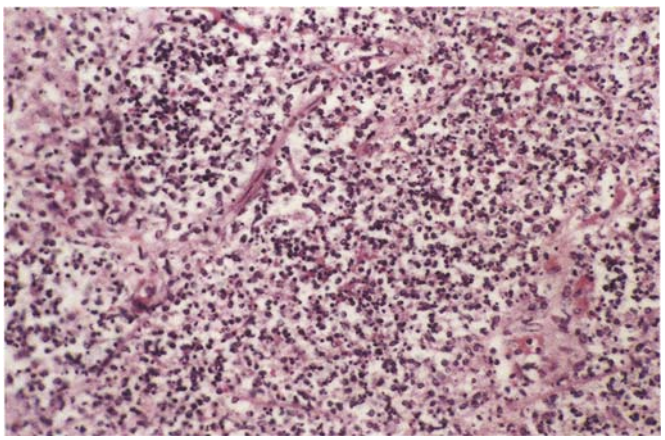

FIGURE 8.62. *Burkholderia cepacia* pneumonia in a patient with cystic fibrosis. There is an intense neutrophilic inflammatory exudates with accompanying necrosis. (Courtesy of Dr. Joseph F. Tomashefski, Jr., MetroHealth Medical Center, Cleveland, OH.)

In a report of surgical pathology and autopsy data from three cases of *B. cepacia* pneumonia in non-CF patients, Belchis and colleagues[578] noted areas of conventional necrotizing pneumonia with abscess formation and fibrin exudates. In contrast to CF patients, however, all three of these cases demonstrated regions of granulomatous inflammation, predominately with central necrosis (Fig. 8.63), within airways or a regional lymph node. Small, nonnecrotizing granulomatous foci were infrequently seen, but hemorrhage, vasculitis, and sarcoid-like granulomas were not identified. Other nonfatal, community acquired infections have also been documented.[579]

A case of chronic progressive pneumonia caused by *B. cepacia* was reported in a patient with chronic granulomatous disease.[132] This group of patients develops atypical granulomatous lesions in response to bacterial pathogens that usually elicit an acute inflammatory reaction.[132] Dailey and Benner,[580] however, reported a case of chronic, necrotizing pneumonia that was caused by *B. cepacia*, then classified as eugonic oxidizer—group 1 (EO-1).

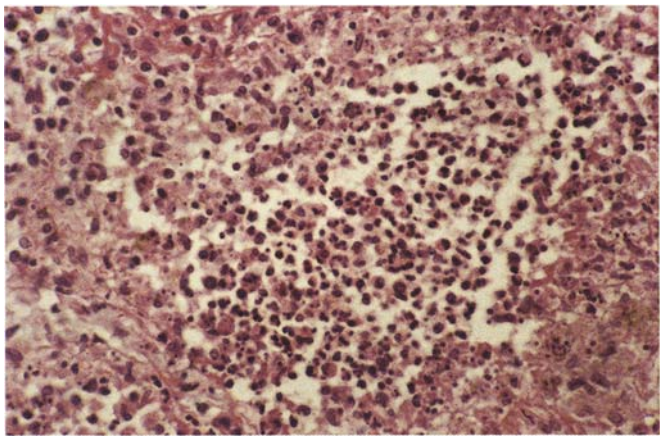

FIGURE 8.63. *Burkholderia cepacia* pneumonia in a patient without cystic fibrosis. There is a focus of granulomatous inflammation with central necrosis and neutrophilic infiltration. (Courtesy of Dr. Joseph F. Tomashefski, Jr., MetroHealth Medical Center, Cleveland, OH.)

Burkholderia pseudomallei

Melioidosis is a geographically limited bacterial disease of protean clinical manifestations first described by Whitmore and Krishnaswami[581] in 1912. It is caused by *Burkholderia pseudomallei* (Whitmore's bacillus; formerly *Pseudomonas pseudomallei*), a small, motile, gram-negative, aerobic bacillus that is a ubiquitous saprophyte of soil, ponds, stagnant water, and rice paddies in tropical regions between 20° north and south latitudes.[582] The disease is endemic in Southeast Asia. Humans as well as wild and domestic animals are susceptible to infection, but it is thought that animals do not serve as a reservoir for human disease. Serologic data indicate that approximately 9% of U.S. soldiers had subclinical infection with *B. pseudomallei* while in Southeast Asia during the Vietnam War.[583] Significant antibody titers, moreover, were demonstrated in up to 50% of native populations within the neighboring country of Thailand during the same time period.[584] More than 300 cases have been encountered in military personnel months to years after their return from Vietnam, because melioidosis often has a long latent period.[585,586]

The usual portals of entry for *B. pseudomallei* are the alimentary tract and abraded or traumatized skin. In addition, primary pulmonary infection can follow inhalation of aerosolized water droplets or dust particles contaminated with the organism.[582] The incubation period is extremely variable, ranging from a few days in the acute pulmonary form of melioidosis up to months and even years in the localized subacute and chronic forms. Human-to-human transmission of infection has been suggested on the basis of serologic data.[587]

Clinically patients with melioidosis can present with an acute localized suppurative infection, acute pulmonary disease, acute septicemia, or a chronic suppurative infection.[582,588–590] The most common clinical form is pulmonary infection, which can be either primary from direct inhalation of the organism or secondary after hematogenous dissemination in the septicemic form. Chest radiographs usually reveal consolidation or nodular densities of the upper lobes. Cavitation, which occurs frequently, can mimic tuberculosis.[582,591] Pleural effusions are uncommon, but a localized pleural mass caused by *B. pseudomallei* has been reported.[592] In chronic suppurative infections, localized lesions occur in the skin, lungs, liver, spleen, lymph nodes, brain, myocardium, bones, and joints.[593–596]

Microscopically the acute form of melioidosis is characterized by multiple discrete abscesses that measure 1 mm to several millimeters in diameter and are most often found in the lungs, liver, and spleen (Fig. 8.64).[588,589,597] The abscesses contain neutrophils, macrophages, fibrin, and giant cells that superficially resemble megakaryocytes. If a patient survives the acute infection, epithelioid histiocytes, lymphocytes, and multinucleated giant cells of both the Langhans' and foreign-body types surround the abscess as the lesion becomes granulomatous.[582,589,591] In long-standing infections granulomas are often encapsulated by dense fibrous connective tissue. The centers of granulomas consist of either stellate abscesses or caseous necrosis that resembles the lesions of tuberculosis. In the lymph nodes stellate abscesses are similar to those seen in tularemia, cat-scratch disease, and lymphogranuloma venereum. Numerous bacilli of *B. pseudomallei* that measure 0.8 × 2.0 μm are readily demonstrated within

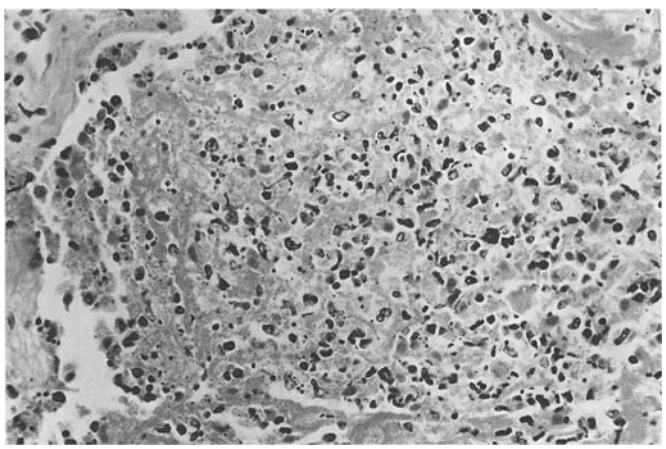

FIGURE 8.64. Pulmonary melioidosis caused by *Pseudomonas pseudomallei.* Masses of fibrin, polymorphonuclear leukocytes, and fewer macrophages fill alveolar spaces at the periphery of the abscess. Exudate is undergoing lysis similar to that seen in *Legionella* pneumonia. Vasculitis, as seen in *Pseudomonas aeruginosa* infections, is absent.

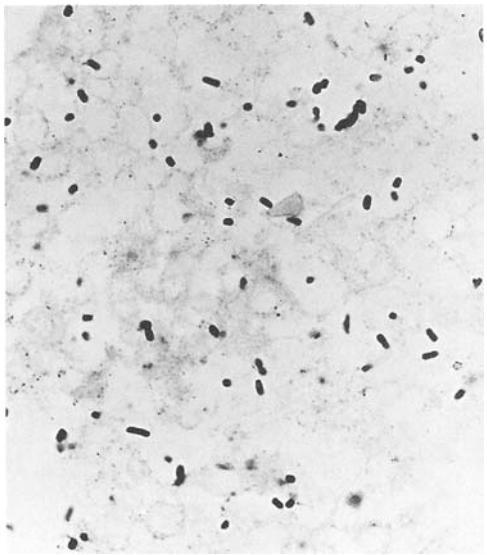

FIGURE 8.65. Small pleomorphic bacilli of *Pseudomonas pseudomallei* within necrotic center of pulmonary granuloma. Few bacilli show bipolar staining. (Steiner silver impregnation method.)

macrophages in acute lesions. The bacilli, however, are very sparse and difficult to detect in the suppurative or caseous centers of the granulomas in chronic lesions (Fig. 8.65). Wong and colleagues[597] proposed that the presence of collections ("globi") of bacilli within multinucleate histiocytes, detected by a tissue Gram stain, in association with a background of necrotizing inflammation is very characteristic, possibly even pathognomonic of *B. pseudomallei* infection. Although the Brown–Hopps and Giemsa stains have been recommended for demonstrating *B. pseudomallei* in tissue sections,[589] organisms are much more easily seen with silver impregnation stains, such as the Warthin-Starry, Steiner, and Dieterle procedures (Fig. 8.65). Alternatively, conventional Gram stain may be applied to a touch preparation of unfixed lung or a scraping of formalin-fixed pneumonic lung.

A diagnosis of melioidosis can be made by microbiologic culture, by serologic testing, or by immunofluorescence staining of *B. pseudomallei* in smears and formalin-fixed deparaffinized tissue sections.[346,582,598] Laboratory-acquired infection with *B. pseudomallei* has been described.[599]

Miscellaneous Pseudomonads

Rosenthal and associates[600] have described a case of pneumonia in a patient who experienced near-drowning. *Pseudomonas putrefaciens* was recovered repeatedly from sputum and from water at the site of the accident. It is the likely cause of the pneumonia, but was not isolated from sterile sites. *Pseudomonas stutzeri* has also been implicated infrequently as the cause of community-acquired pneumonia.[601] *Stenotrophomonas maltophilia* (formerly *Pseudomonas* and *Xanthomonas maltophilia*) has been isolated from aspirated bronchial secretions of a patient with pneumonia, whose disease responded to therapy only when an effective antibiotic was employed for this multiply resistant bacterium.[602] This species has been recognized as a cause of nosocomial pneumonia,[603,604] but geographic and environmental factors determine its importance. The respiratory tracts of many patients are colonized without resultant infection.[605,606] Trotter and associates[607] reported a case of pneumonia caused by an unclassified pseudomonad that phenotypically resembled *B. cepacia.*

Moraxella *spp.*

Moraxella species are gram-negative coccobacilli that may resemble *Neisseria* species morphologically. The most important human pathogen, *Moraxella catarrhalis*, has the appearance of a diplococcus and was previously classified as *Neisseria catarrhalis*, then as *Branhamella catarrhalis.* Recognition of *M. catarrhalis* as a cause of community-acquired and nosocomial respiratory tract infections, including pneumonia and bronchitis, was delayed, because it is frequently a part of the resident oropharyngeal flora.[608,609] The clinical illness is rarely life threatening, and bacteremic infection is rare.[610,611] Wright and colleagues[612] summarized the characteristics of *M. catarrhalis* pneumonia. Most patients have a serious underlying disease, including immunoglobulin deficiencies or malignancy.[130,131,613] Many patients are elderly and have chronic obstructive lung disease, but infection in children and neonates has also been described.[614,615] The radiographic infiltrates are typically described as patchy with focal consolidation. In as many as half the patients, the infiltrates have an interstitial appearance that suggests pulmonary edema. Bacteremia occurs rarely,[616,617] but abscess formation and empyema have not been described. Most *Moraxella* species are susceptible to the action of penicillin and other β-lactam antibiotics. *M. catarrhalis*, however, frequently produces β-lactamase and may not respond to therapy with β-lactam antibiotics even if standard tests indicate susceptibility.[615] For an excellent comprehensive review of this bacterium, the reader is referred to a recent publication by Verduin and colleagues,[613] who have reviewed *M. catarrhalis* and the associated infections.

Rosett and colleagues[618] described the case of a 78-year-old man with an indolent pneumonia, productive of purulent sputum. Chest radiographs demonstrated a patchy pneumonia in the left-lower lobe and consolidation of the superior segment. Infiltrates later appeared in the right lung, and an abscess was noted in the

consolidated segment of the left lung. *Moraxella nonliquefaciens* was recovered from sputum and a transtracheal aspirate. A case of pneumonia in a renal transplant patient has been attributed to a *Moraxella*-like bacterium that most closely resembled CDC group M-5, which is usually associated with dog bites.[619]

Flavobacterium *spp. and Related Organisms*

Flavobacteria are gram-negative bacilli, often yellow pigmented, that are frequently found in the environment. Most infections have occurred in neonates, in whom *Flavobacterium meningosepticum* may produce a devastating meningitis,[620] but meningitis may occur in adults also.[621] Only a few cases of pneumonia have been attributed to *F. meningosepticum.* Ashdown and Previtera[622] reported a community-acquired pneumonia in a 76-year-old man who had *a F. meningosepticum* septicemia. The authors commented on the similarity of the clinical presentation to melioidosis. Sundin and colleagues[623] described a previously healthy 5-year-old girl in whom pneumonia developed as part of a disseminated infection. At autopsy there were bilateral bronchopneumonia, bronchiolitis, and focal hyaline membranes; further details were not given. Tam and colleagues[624] described a 2-week-old infant with primary nosocomial pneumonia. At autopsy there were diffuse consolidation and focal hemorrhage. Microscopically extensive denuding of the bronchiolar and bronchial epithelium occurred. Polymorphonuclear leukocytes accompanied hemorrhage and hyaline membranes in the air spaces. A mixed inflammatory infiltrate was present in the interstitium. Nosocomial pneumonia has also been reported in adults,[625] including an outbreak associated with aerosolized polymyxin B.[626]

One of the peculiarities of *F. meningosepticum* is resistance to antibiotics that are usually employed for gram-negative infections and susceptibility to certain antibiotics that are ordinarily reserved for gram-positive bacteria, such as rifampin and vancomycin.[627]

Casalta and colleagues[628] described an unidentified gram-negative bacterium that resembled *Flavobacterium* sp. The pulmonary process was described as lobar radiographically, but no further details were given.

Vibrionaceae

The family Vibrionaceae includes the genera *Aeromonas* and *Vibrio.* Both these genera consist of fermentative gram-negative bacilli that contain indophenol oxidase. They are environmental aquatic organisms that most commonly produce gastroenteritis or wound infection, depending on the species.[37]

Several cases of *Aeromonas hydrophila* pneumonia have been reported. Reines and Cook[629] described three patients with pneumonia and sepsis. One patient was immunocompromised, and the two normal individuals had experienced near-drowning. At autopsy of the one fatal case there was a necrotizing pneumonia with abscesses. Baddour and Baselski[630] reported eight cases of *Aeromonas* pneumonia and reviewed other cases from the literature. All patients had serious underlying diseases, and an episode of aspiration was documented in six individuals. Once again, the two healthy patients had experienced near-drowning episodes.

Kelly and Avery[631] reported a case of pneumonia and septicemia caused by *Vibrio vulnificus* (formerly lactose-positive *Vibrio*) in a previously healthy man who was found floating face down in the sea. This species has been responsible for serious wound infections with a high mortality rate.[632]

Acinetobacter *Species*

Acinetobacter includes five species of which the most important is *Acinetobacter baumanii.* The taxonomy and nomenclature of this genus have been a kaleidoscopic tapestry rivaling that of non-Hodgkin's lymphoma. Previous names include *Acinetobacter anitratum, Acinetobacter calcoaceticus* var. *anitratus*, and *Herrelea vaginicola.*[37]

Acinetobacter baumannii has been described as a cause of community-acquired pneumonia in patients with serious underlying disease,[633–635] of epidemic pneumonia in an industrial setting,[636] and of epidemic nosocomial pneumonia associated with contaminated respirometers.[637] All patients have been immunocompromised or afflicted with serious underlying diseases. The pneumonia has been described as focal, occasionally with confluence. Microscopically there is an air-space exudate of polymorphonuclear neutrophils and macrophages. Abscesses may develop. Bacteria are usually demonstrable in the lesions, but this very short gram-negative bacillus may be misidentified as a gram-negative coccus.[633]

Pasteurella *spp.*

All the major pathogens have been transferred from the genus *Pasteurella* to other genera, such as *Yersinia* and *Francisella.* The species most commonly isolated from human specimens is *Pasteurella multocida.* This species is commonly found in animals and often causes wound infections after dog or cat bites, but colonization and infection of the respiratory tract occur in the absence of exposure to animals.[37] Most cases of *P. multocida* pneumonia have occurred in patients with chronic bronchitis or a history of aspiration.[638–640] The pneumonia is usually described as patchy and focal. The bacteria may be pleomorphic, resembling *Haemophilus*, or coccobacillary, even resembling *Neisseria* spp. Necrotizing infection with abscess formation and empyema may occur rarely.[641–643]

A case of focal pneumonia attributed to *Pasteurella ureae* has been described. The infection was acquired in the hospital and was documented by culture and Gram stain of tracheal aspirates.[644]

Francisella tularensis

Tularemia is a systemic bacterial infection caused by the small, gram-negative, pleomorphic bacterium *Francisella tularensis.* The disease, which is endemic in ground animals, particularly rabbits, rodents, squirrels, cats, and raccoons, is sometimes transmitted to humans through handling these animals.[645,646] Insect vectors, especially ticks, can also be the source of infection.[645–647] Most cases of tularemia, therefore, occur in rural areas, but suburban residents are also at risk.[648,649]

The principal clinical forms of tularemia are ulceroglandular, pneumonic, oculoglandular, and typhoidal.[650] Pulmonary involvement is present in most fatal cases of tularemia and may represent either the primary or secondary form of the disease.[651–653] Pneumonia may result from septicemia or from inhalation of aerosolized infectious droplets. An outbreak of primary pneumonic tularemia on Martha's Vineyard, Massachusetts, was epidemiologically linked to mowing lawns and cutting brush in presumed rabbit habitat.[654] Although most patients with tularemia survive the disease, pulmonary involvement usually portends a poor prognosis.[651–653] Sunderrajan and colleagues[655] reported three patients who developed the clinical picture of adult respiratory distress syndrome with diffuse parenchymal infiltrates as a part of *F. tularensis* pneumonia.

Macroscopically the lungs from patients who die of tularemia with pulmonary involvement exhibit a multifocal pneumonia. The areas of pneumonia may be confluent, resulting in a lobar pattern of consolidation. Multiple small abscesses also may be present. Microscopically abundant fibrin is present in alveoli, and the cellular component of the exudate consists primarily of mononuclear cells (Fig. 8.66). As the disease progresses, parenchymal necrosis develops. The necrotic areas may resemble infarcts or geographic foci of caseation.[656,657]

The vascular changes in acute tularemic pneumonia may be quite pronounced, including extensive thrombosis and necrosis of small and medium-sized arteries and veins (Fig. 8.67). Although small foci of granulomatous inflammation may be seen in tularemia, giant cells are usually absent. *F. tularensis* stains poorly with tissue Gram stains and is very difficult to demonstrate in histologic sections (Fig. 8.68). When present, the bacilli are usually within macrophages and epithelioid histiocytes. Although only faintly Gram negative, the bacterium is intensely argyrophilic when stained with the Steiner, Dieterle, or Warthin–Starry silver impregnation procedures. Diagnosis of the disease requires the bacteriologic isolation of the causative organism; demonstration of a specific immunologic response; detection of the organism by direct immunofluorescence in smears of lesional exudate or in formalin-fixed, deparaffinized tissue sections (Fig. 8.69); detection of antigen by enzyme immunoassay or latex agglutination; or demonstration of species-specific DNA by molecular amplification techniques.[346,649,658,659] Roy and colleagues[660] reported cross-reactions between *L. pneumophila* and *F. tularensis* in the direct immunofluorescence test. Further, *F. tularensis* may be isolated on buffered charcoal yeast extract agar, the medium normally used for culture of *Legionella* spp.[661] Both genera require cysteine in the medium for

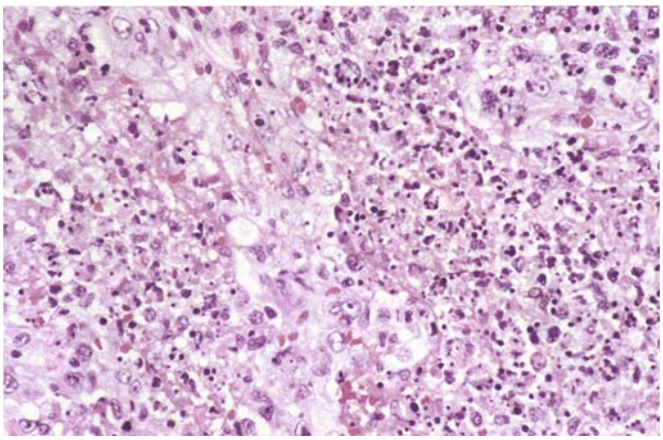

FIGURE 8.66. Tularemic pneumonia. Abundant fibrin, macrophages, and degenerated neutrophils fill alveoli and bronchioles. Note necrosis of alveolar septa.

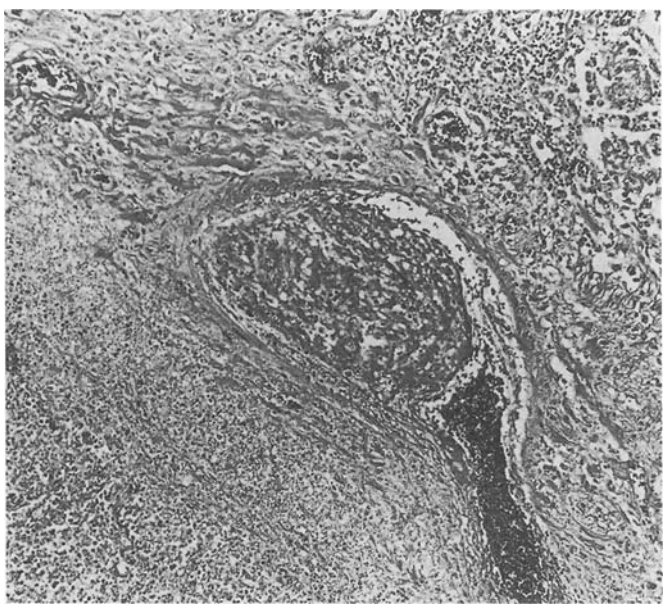

FIGURE 8.67. Vascular thrombosis in tularemic pneumonia.

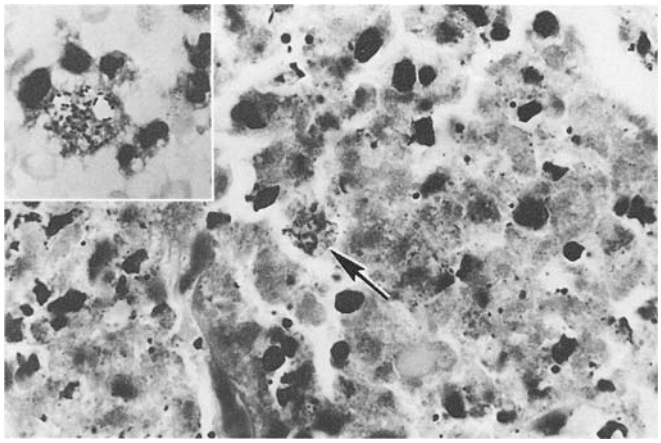

FIGURE 8.68. Tularemic pneumonia. Alveolar macrophages contain rare, minute, weakly gram-negative coccobacilli (arrow). (Brown–Hopps procedure.) Inset: Macrophage contains numerous pleomorphic coccobacilli of *Francisella tularensis* in touch preparation of fresh lung. (Giemsa stain.)

optimal isolation. *Legionella* spp. do not present a biohazard in the laboratory under normal conditions, but *F. tularensis* must be handled with great care, because acquisition by laboratory personnel is a well-known occupational risk.[662]

Brucella *spp.*

Brucellosis is a zoonotic disease caused by six species of small, gram-negative, nonmotile, and non–spore-forming coccobacilli in the genus *Brucella*.[663,664] Humans are infected by direct contact with tissues and body fluids of chronically infected animals, especially cattle, goats, sheep, and swine, or by ingestion of raw milk, milk products, and tissues contaminated with the bacterium.[665–667] *Brucella* spp. gain entrance into the body via the skin, mucous membranes, alimentary tract, and lungs. Individuals who have close, continuous contact with domestic animals are at increased risk of infection. Most human infections are caused by four species: *Brucella abortus*, from cattle; *Brucella melitensis*, from goats and sheep; *Brucella suis*, from swine; and *Brucella canis*, from dogs.[666,668] In the U.S. and Canada, most human infections are caused by *B. abortus*, whereas *B. melitensis* and *B. suis* are the most common etiologic agents in other countries. The three major clinical forms of brucellosis are acute malignant infection, relapsing or undulant fever, and intermittent or chronic disease.[663,664,667]

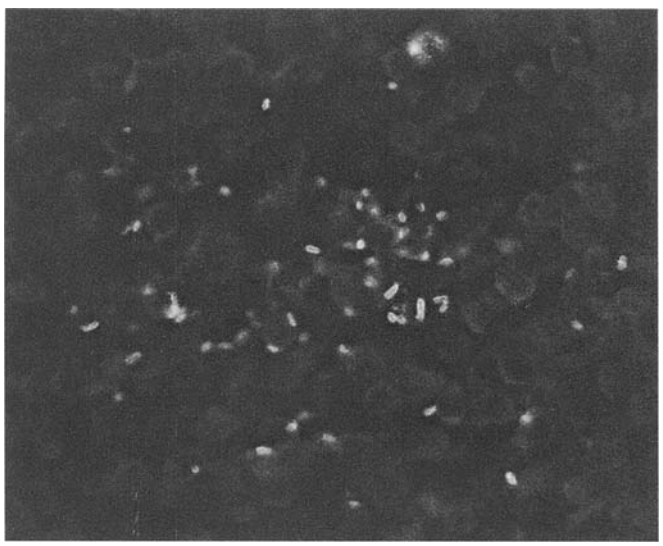

FIGURE 8.69. Tularemic lymphadenitis. Minute, brightly fluorescent coccobacilli in center of abscess. Formalin-fixed deparaffinized section was stained with fluorescein-conjugated antiglobulins specific for *Francisella tularensis*.

Following entry into the body, the *Brucella* spp. disseminate via the mononuclear phagocyte system. As facultative intracellular pathogens, they multiply predominantly within monocytes and macrophages.[663,669] Generalized hyperplasia of the mononuclear phagocyte system usually results in lymphadenopathy and hepatosplenomegaly in about half of infected patients. In the subacute stage of infection patients may also develop noncaseating epithelioid cell granulomas and lymphocytic infiltrates in the lymph nodes, spleen, liver, bone marrow, synovial membranes, meninges, genitourinary tract, and lungs.[669–674] Acute bacterial endocarditis has also been described, as have multifocal abscesses in the myocardium.[669,675] Intracellular gram-negative coccobacilli are more easily demonstrated in the acute and subacute lesions than in the more chronic stages. The organism can usually be isolated from the blood and infected tissues in all forms of brucellosis, however. In our experience, silver impregnation stains (Steiner, Dieterle or Warthin-Starry) have been superior to the Brown-Hopps or other tissue Gram stains for demonstrating sparse intracellular organisms in histologic sections (Fig. 8.70).

In chronic brucellosis, the organs mentioned previously often contain solitary or multiple, sharply outlined, caseous or suppurative granulomas that resemble those seen in tuberculosis.[669,676] Residual fibrocaseous nodules or coin lesions that resemble tuberculomas, histoplasmomas, or coccidioidomas in H&E-stained sections have been described in the lungs.[676] More typical patterns of pulmonary involvement, including lobar and interstitial disease, have been recognized.[677,678] Pleural effusions have also been described.[677,678]

A diagnosis of brucellosis can be confirmed by microbiologic culture of blood and tissue specimens, by serologic procedures, by molecular amplification methods, or by immunofluorescence staining of the bacterium in

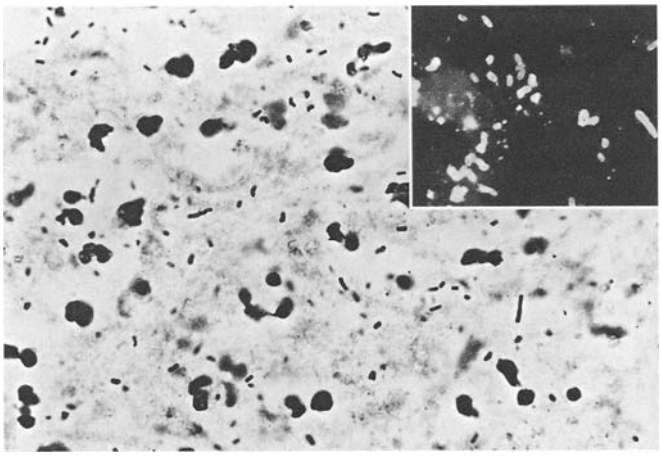

FIGURE 8.70. Chronic pulmonary brucellosis. Small bacilli and coccobacilli seen at the margin of a caseous granuloma. Inset: In replicate deparaffinized section, bacilli are intensely decorated with immunofluorescence conjugate specific for *Brucella* spp. (to genus level only).

smears or formalin-fixed deparaffinized tissue sections (Fig. 8.70 inset).[346,679–682]

Bordetella spp.

Whooping cough was one of the diseases that effective immunization practices had virtually eliminated, but changes in those practices have led to a resurgence of disease in recent decades.[683] The pertussis syndrome is most commonly caused by *Bordetella pertussis*, a tiny gram-negative coccobacillus.[684,685] *Bordetella parapertussis* may cause a milder syndrome.[686] The clinical syndrome includes an early catarrhal phase, followed by a period of laryngotracheobronchitis, a phase of characteristic paroxysmal cough, and finally a recovery phase. Atypical lymphocytosis may occur, but is inconstant. The differential diagnosis includes viral infection of the lower respiratory tract.[687] In fact, whooping cough and viral infection may coexist in young children (see Fig. 11.5G in Chapter 11).[688]

Most cases of whooping cough occur in nonvaccinated infants, but cases have been reported in adults,[689] including those with waning immunization.[690,691] Infections in adults and immunized patients tend to be mild. *B. pertussis*, which is found only in humans, is maintained in nature by person-to-person spread, usually with mild or subclinical infection resulting.[690] *B. pertussis* has been isolated from patients with AIDS and respiratory infections.[692]

The clinical diagnosis of pertussis is imprecise,[693] and laboratory support is important. The primary diagnostic modality is bacterial culture,[694] but the bacterial colonies require several days to develop. Bordet-Gengou medium, the traditional choice, has been replaced or supplemented by a charcoal-based medium.[695] Direct immunofluorescence of the bacteria in clinical specimens provides a more rapid diagnosis,[696,697] but this method is plagued by insensitivity and nonspecificity.[528,694,698] Serologic diagnosis is particularly useful for mild cases[690,699] but is perforce retrospective and is not readily available in this country. Recent use of molecular amplification procedures for detection of *B. pertussis* has improved diagnostic sensitivity dramatically.[700,701]

Most cases of whooping cough are not fatal, and there is little published information on the pathology of the infection. As suggested by the clinical symptoms, the site of injury is primarily in the airways rather than in the alveoli themselves. The lymphocytic inflammatory exudate infiltrates the submucosa of the large airways (Fig. 8.71) and all layers of distal bronchioles (Fig. 8.72). Toxic effects on the epithelial cells are difficult to differentiate from postmortem effects. *B. pertussis* is most readily cultured during the early, unfortunately nonspecific, catarrhal phase of the illness. Recovery of the bacteria is increasingly difficult as the infection progresses and demonstration of the organism in tissue is usually not accomplished. The differential diagnosis of the pathologic lesions, as well as the clinical symptoms, is viral infection, particularly respiratory syncytial virus, adenoviruses, and parainfluenza viruses (see Chapter 11).

Bordetella bronchiseptica is a pathogen of animals.[702] This easily cultivated species may also cause chronic bronchitis and has been suggested as a cause of pneumonia in a patient who may have acquired the infection from his dog.[703] The organism has also been implicated in several cases of pulmonary infection in patients with some degree of immunosuppresion.[704–706]

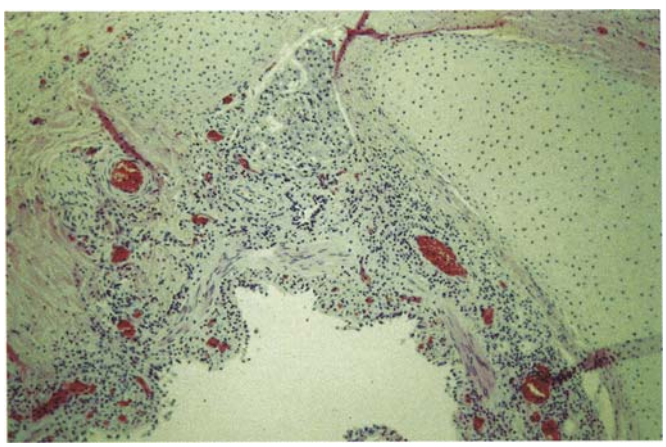

FIGURE 8.71. *Bordetella pertussis* bronchitis. Infant girl, who had not been immunized for religious reasons, died of whooping cough. Bronchial mucosa is extensively infiltrated with mononuclear cells; epithelial cells are largely sloughed. *Bordetella pertussis* was isolated in pure culture from the lung postmortem. (Courtesy of Dr. Paul Morrow.)

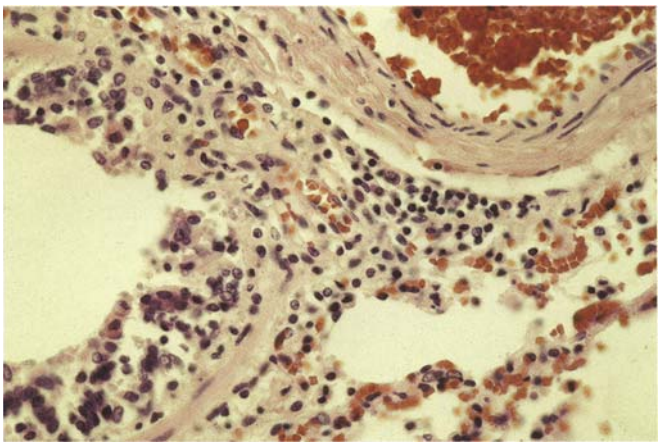

FIGURE 8.72. *Bordetella pertussis* bronchiolitis. Bronchiolar mucosa and submucosa and pulmonary interstitium from case shown in Figure 8.71 is infiltrated with mononuclear cells. Mucosa is focally sloughed.

Miscellaneous Gram-Negative Bacilli

Several cases of bacteremic pneumonia caused by *Alcaligenes xylosoxidans* (formerly *Achromobacter xylosoxidans*) have been described.[707,708] In each case there was a serious underlying disease. Details of the pathologic processes were not given.

Eikenella corrodens is a facultatively anaerobic, gram-negative bacterium that is commonly a part of the microflora of the oropharynx. The bacterium gets its name from the distinctive manner in which the colonies "pit" the agar medium, producing a dewdrop or fried egg appearance.[37] Goldstein and colleagues[709] described the recovery of this organism from respiratory secretions of 16 patients, seven of whom had pneumonia or lung abscess. All seven patients had serious underlying disease and four had carcinomas. In all seven cases *Eikenella* was isolated as part of a polymicrobial infection. The nature of the pulmonary lesions was not detailed.

Obligately Anaerobic Bacteria

Most anaerobic infections of the lung are polymicrobial and follow aspiration of oropharyngeal contents, where anaerobes constitute a majority of the normal flora. The most distinctive manifestations of anaerobic pleuropulmonary infections are thoracic actinomycosis, putrid lung abscess, and empyema. A foul or fecal odor in a clinical specimen suggests an anaerobic component to the infection. Certain anaerobes, such as *Peptostreptococcus anaerobius* and some *Bacteroides* species, have a very disagreeable odor even on agar plates. Not all anaerobic bacteria produce odoriferous end products, however. Actinomycotic infections should not be expected to smell bad.

Actinomyces *spp. and Related Bacteria*

Actinomycosis is a sporadic localized infection of worldwide distribution caused by anaerobic or microaerophilic filamentous bacteria in the order Actinomycetales.[306,710–712] The disease is not contagious, and its causative agents have never been isolated from any natural habitats outside the body. The agents of actinomycosis occur as commensals of the mouth, throat, gastrointestinal tract, and vagina of healthy individuals.[306,711–713] These endogenous microorganisms are opportunists that have the capacity to invade injured tissues and intraabdominal mucosal breaks. Unlike nocardiosis, actinomycosis does not occur preferentially in immunocompromised patients. Because actinomycosis is an endogenous disease, it is not included under mycetoma, despite the fact that its etiologic agents commonly form granules in tissue. The principal agent of actinomycosis in humans is *Actinomyces israelii.* Other causes of the disease are *Actinomyces naeslundii,*[714–716] *Actinomyces viscosus,*[716–720] *Propionibacterium propionicus* (formerly *Arachnia propionica*),[721,722] and, rarely, *Actinomyces odontolyticus,*[723–725] *Actinomyces meyeri,*[726,727] *Eubacterium nodatum,*[728] and *Rothia dentocariosa. Actinomyces* spp. and related bacteria may be part of a mixed anaerobic infection, and there is experimental evidence that other bacteria may enhance the pathogenicity of the actinomycetes.[729]

Based on the anatomic site of infection, most cases of actinomycosis are classified as cervicofacial, thoracic, abdominal, or pelvic. Cervicofacial infection or "lumpy jaw" is the most common clinical form. It may develop without any known antecedent injury to the oral mucosa.[710,730] Frequently, however, cervicofacial actinomycosis follows a dental extraction. Less commonly it is a sequel to dental caries, periodontal disease,[716] or an accidental injury to the oral mucosa. Infected tissues are swollen, firm, and elastic. As the disease progresses, abscesses form and draining sinus tracts emerge. If untreated, the infection may extend upward to involve the sinuses, the orbit, or the cranial bones.

Thoracic actinomycosis usually results from aspiration of infectious materials, but it may also develop by direct extension of a cervicofacial infection.[712,726,731–733] Patients have either isolated lung disease or a combination of lung and chest wall involvement. Clinically, symptoms of thoracic actinomycosis often suggest a malignancy.[734,735] Abdominal actinomycosis can develop by direct extension of a thoracic infection but more commonly results from a ruptured appendix or penetration of the etiologic agent through the wall of the stomach or intestines.[710,736]

Thoracic actinomycosis represents approximately one fifth of all cases and is characterized by chest pain, fever, chills, night sweats, and weight loss.[737] Chest radiographs usually reveal pulmonary consolidation, numerous small opacities with cavitation, and pleural thickening.

Osteomyelitis of adjacent ribs may also be present.[738–741] Macroscopically lungs infected with the agents of actinomycosis exhibit scarring and multiple abscesses 0.1 cm to several centimeters in diameter. The lung is often adherent to the parietal pleura, and there may be inflammation in the soft tissues of the chest wall. Sinus tracts discharging to the skin are occasionally seen, and sinus exudates may contain sulfur granules.[742,743] The diagnosis is generally made by histologic examination of surgical specimens because the responsible agents are anaerobic and difficult to culture from lung. Actinomycosis is usually not suspected before surgery.[744] Surgical drainage and excision of diseased tissues are also important in the treatment of this disease.[744]

The suppurative reaction to the agents of actinomycosis is characterized by the formation of abscesses that contain one or more actinomycotic granules and are encapsulated by fibrosing granulation tissue (Fig. 8.73).[306] In most cases the granules are bordered by intensely eosinophilic, club-like projections of Splendore–Hoeppli material. Granules range from 30 to 3000 μm in diameter and can sometimes be seen with the naked eye when a stained tissue section is held up to the light.[306,710,742] Abscesses vary in size, may be solitary or multiple, and are characteristically surrounded by numerous histiocytes that have foamy cytoplasm because of their lipid content.

A modified Gram stain, such as the Brown and Brenn procedure, reveals that each granule is composed of delicate, branched, gram-positive, and often beaded filaments approximately 1 μm in width that are embedded in an amorphous matrix of uncertain composition.[306,329,743] Branching usually occurs at right angles. In some granules the filaments are radially oriented at the periphery, and scattered rods and coccoid forms may also be detected, as demonstrated by the Grocott methenamine silver technique (Fig. 8.74). In H&E-stained tissue sections entire granules with their eosinophilic, serrate borders of Splendore–Hoeppli material are easily delineated and represent the sulfur granules classically described in actinomycosis.[306,329] Individual actinomycetal filaments that form the granule are not visible, however. The Grocott methenamine silver and modified Gram stains are required to demonstrate actinomycetes in tissue sections; Gridley and PAS procedures do not reliably stain these microorganisms. Although the filaments of *Actinomyces* spp. and related bacteria are not acid fast, the Putt procedure for demonstrating partial acid-fastness in tissue may occasionally produce false-positive results.[197,329] The Fite or Fite–Farraco procedure will demonstrate the appropriate staining reaction.

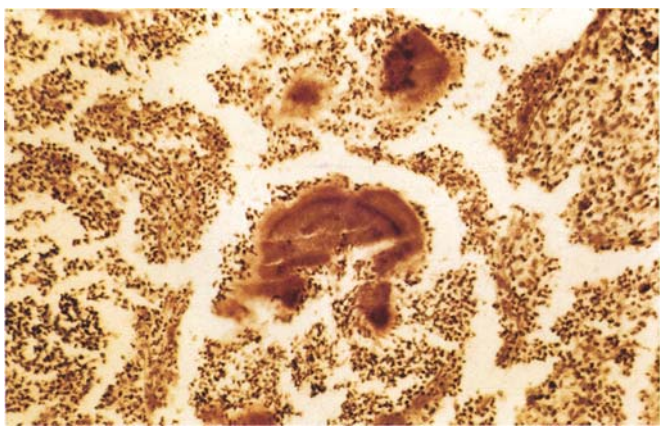

FIGURE 8.73. Pulmonary actinomycosis caused by *Actinomyces israelii*. Several irregular granules are embedded in abscess and surrounded by Splendore–Hoeppli material. Individual actinomycetes within the granules cannot be distinguished at this magnification. (Brown-Hopps procedure.)

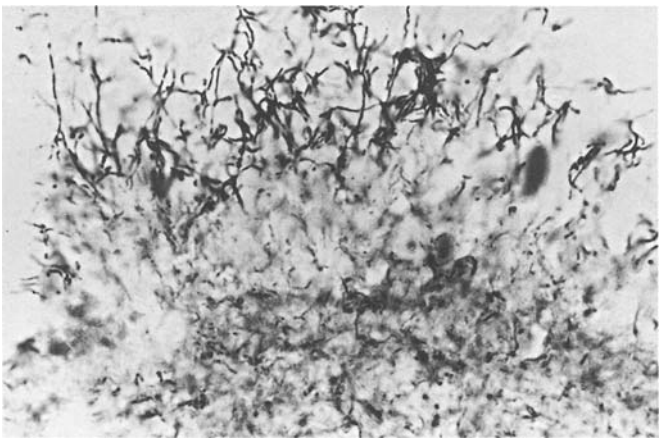

FIGURE 8.74. Actinomycosis caused by *Actinomyces israelii*. Beaded bacterial filaments stained by Grocott's methenamine silver procedure branch predominantly at right angles and are radially oriented at periphery of granule. (Grocott's methenamine silver procedure.)

Behbehani and colleagues[745] described morphologic differences among lesions produced by various *Actinomyces* spp. in experimentally infected mice. The granules of *Actinomyces israelii* exhibited a peripheral eosinophilic fringe without penetration of polymorphonuclear neutrophils into the granule, and the lesions were still progressing 6 weeks after initiation of infection. In contrast the lesions produced by *Actinomyces naeslundii* and *Actinomyces viscosus* were resolving 6 weeks after infection, and the granules were penetrated to varying degrees by polymorphonuclear neutrophils. The granules of *A. viscosus* showed considerable disintegration, whereas those produced by *A. naeslundii* remained relatively intact.

The agents of actinomycosis can be definitively identified by isolating and identifying them in culture. They

grow best at 37°C on enriched, antibiotic-free media where they form raised, whitish colonies that are either smooth or rough depending on the amount of filamentation. Definitive identification of *Actinomyces* and related bacteria also can be achieved by immunofluorescence staining of clinical specimens and deparaffinized sections of formalin-fixed tissue.[746,747]

The major problems in the histopathologic differential diagnosis of actinomycosis are botryomycosis caused by nonfilamentous bacteria and actinomycotic mycetoma caused by the exogenous *Nocardia* spp. Differentiation is important because each of these diseases is managed differently. Botryomycosis is a chronic, localized bacterial pseudomycosis usually of the skin and subcutaneous tissues. It is caused by gram-positive and gram-negative bacteria that form compact aggregates or granules.[306] These granules are almost always bordered by Splendore–Hoeppli material, and, like those of actinomycosis, are found within encapsulated abscesses. In H&E-stained tissue sections, botryomycotic granules are usually indistinguishable from those of actinomycosis because the bacteria that form these granules are poorly stained. Histopathologic differentiation is achieved with tissue Gram stains, such as the Brown–Hopps and Brown and Brenn procedures, which demonstrate bacterial cocci and bacilli in botryomycosis or delicate, branched, gram-positive filaments in actinomycosis.

Rarely, *Nocardia* spp. can cause actinomycotic mycetoma with the formation of granules that are morphologically similar to those of actinomycosis in tissue sections stained with H&E, modified Gram, and Grocott's methenamine silver procedures.[306,329] Unlike the agents of actinomycosis, however, *Nocardia* spp. are weakly acid fast when stained with modified acid-fast procedures that use an aqueous solution of a weak acid for decolorization.

Aspiration Pneumonia

The bacteriology of aspiration pneumonia has been well described.[36,224] Obligately anaerobic bacteria are recovered in more than 90% of these cases and are the only isolates in approximately half of cases. The infections are almost always polymicrobial. The anaerobic species most commonly isolated are *Prevotella melaninogenica* (formerly *Bacteroides melaninogenicus*), *Fusobacterium nucleatum*, and anaerobic or microaerophilic gram-positive cocci. The same group of bacteria also causes oropharyngeal infections, such as peritonsillar abscess, and occasionally anaerobic lung infection may accompany infection of the upper airways.[748]

Although *Bacteroides fragilis* is infrequently isolated from the oropharynx, this penicillin-resistant species was isolated from 17% of aspiration pneumonias.[224] Gram-negative aerobes and facultative anaerobes, such as *P. aeruginosa* and enteric bacilli, were common only in nosocomial infection. A similar group of pathogens is responsible for empyema.[749] Morgenstein and colleagues[750] reported a case of cavitary pneumonia in a 46-year-old man with acute lymphoblastic leukemia in which *Leptotrichia buccalis*, a member of the family *Bacteroidaceae*, was isolated from blood cultures.

Bartlett[751] described the clinical features of 46 patients with anaerobic bacterial pneumonia. Although virtually diagnostic when present, putrid sputum was noted in only two individuals. There were very few differences between the patients with anaerobic pneumonia and a comparison group with pneumococcal pneumonia. Those with anaerobic pneumonia developed chills less frequently and developed abscesses more commonly than those with pneumococcal infection. The average age of patients was 53 years, but anaerobic pneumonia has been described in neonates.[752]

Aspiration pneumonia is often associated with necrotizing pneumonia (Fig. 8.75) and lung abscess, probably because of the enzymes produced by many anaerobic bacteria (see also Chapter 5).

Clostridium species also produce pneumonia, although less frequently than do anaerobic gram-negative bacilli. Necrotizing pneumonia and empyema caused by *Clostridium perfringens* has been reviewed by Bayer and associates.[753] Most patients had underlying lung disease, and aspiration of oropharyngeal contents was believed to be the mechanism of infection. In contrast to those with clostridial myonecrosis, patients with clostridial

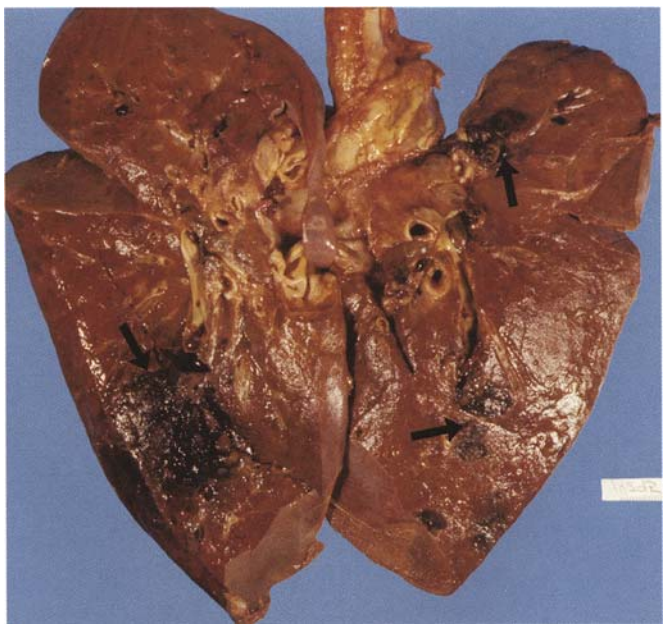

FIGURE 8.75. Aspiration pneumonia caused by *Peptostreptococcus* sp. with multiple large foci (arrows) of necrotizing, hemorrhagic pneumonia.

pneumonia were rarely toxic. Cases of necrotizing pneumonia and empyema caused by *Clostridium sordellii*[754] and by *Clostridium bifermentans* in conjunction with *Bacillus cereus*[372] have been reported.

Botryomycosis

Botryomycosis (bacterial pseudomycosis) is a chronic localized and progressive infection caused by nonfilamentous bacteria that characteristically form grains or granules in infected tissues.[755–758] Clinically, botryomycosis is similar to actinomycosis and actinomycotic mycetoma.[759] Patients typically present with localized indurated inflammatory masses. Draining sinuses usually involve the skin and soft tissues of exposed surfaces such as the hands, feet, and head. Infection often spreads locally to involve contiguous skeletal muscle and bone, but hematogenous or lymphatic dissemination is uncommon. Visceral botryomycosis is rare and usually occurs without concomitant cutaneous or subcutaneous infection.[760–762] The lungs, liver, heart, brain, prostate gland, kidneys, and intraabdominal space are most frequently involved. Pulmonary botryomycosis can be either primary or secondary. The upper lobes are involved more frequently than are the lower lobes.[763,764] Clinical findings include dyspnea, pleuritic chest pain, cough, fever, and hemoptysis. The principal agent of botryomycosis is *S. aureus.* Agents less frequently encountered include *P. aeruginosa, E. coli, Actinobacillus lignieresi, Neisseria mucosa*, and species belonging to the genera *Bacillus, Bacteroides, Proteus*, and *Streptococcus.*[755–758,762,765]

In lesions and purulent exudates that drain from sinus tracts, botryomycotic granules appear as soft, yellow or white, organized bacterial aggregates (microcolonies) that range from 0.2 to 2.0 mm in diameter. One or more granules are embedded in an abscess that is usually surrounded by epithelioid histiocytes, multinucleated giant cells, and fibrous connective tissue. The bacteria within botryomycotic granules are usually hematoxylinophilic, are embedded in an amorphous eosinophilic matrix or ground substance, and are intimately surrounded by brightly eosinophilic, radiating clubs of Splendore-Hoeppli material (Fig. 8.76). The bacterial cocci or bacilli that form the granules are best demonstrated with modified Gram stains, such as Brown-Hopps (best for gram-negative bacteria) or Brown and Brenn (best for gram-positive bacteria). The peripheral Splendore-Hoeppli material is gram negative. A presumptive histopathologic diagnosis can be confirmed by culture.

The pathogenesis of granule formation in botryomycosis is poorly understood. Brunken et al.[766] postulated that either defective host resistance or infection by bacteria with attenuated virulence may be important factors in this localized disease. There have been several recent reports of immunodeficiency states, including chronic granulomatous disease, immunoglobulin deficiencies, steroid therapy, and AIDS associated with botryomycosis.[762,764,765,767] Botryomycosis has also been reported to be a complication of cystic fibrosis,[767] diabetes mellitus,[763] and extensive follicular mucinosis.[768] Of the 10 reported cases of pulmonary botryomycosis, seven have been in children with cystic fibrosis.[763,769]

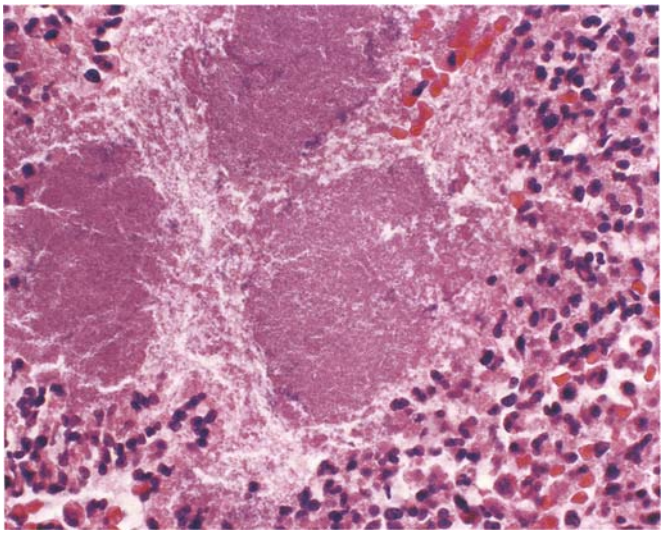

FIGURE 8.76. Botryomycosis. These typical botryomycotic granules are hematoxylinophilic, and embedded in an amorphous eosinophilic matrix. The granules (three shown here) are characteristically surrounded by brightly eosinophilic, radiating clubs of Splendore-Hoeppli material.

Miscellaneous Infections

Malakoplakia

Malakoplakia is an unusual disease of poor macrophage digestion. It was first described in the early years of the 20th century by Michaelis and Gutmann[770] and by von Hansemann,[771] who chose the name because of the soft yellow tumor-like plaques in the bladder of a man with tuberculosis. An excellent general review of this entity has been provided by Damjanov and Katz.[772]

The disease is most common in the bladder, but can occur almost anywhere. It can be confused with malignant tumor masses wherever it occurs. Seventy-five percent of cases are caused by *E. coli*, but other gram-negative and gram-positive bacteria, fungi, and even mycobacteria have been involved.

A small number of cases have been described in the lung. Multiple lower-lobe nodules that seemed to center on bronchi were present in the first case reported by

Gupta et al.[773] in 1972. Subsequently two cases were reported by Colby and associates[774] in immunosuppressed hosts. Their first case occurred as a persistent left upper lobe nodule 3 years after heart transplantation; malakoplakia in this case was diagnosed by needle aspiration and confirmed at autopsy. Their second case occurred in a setting of recurrent Hodgkin's disease, was endobronchial, and obstructed the right middle lobe; the diagnosis was made by transbronchial biopsy. In 1984 Hodder et al.[775] reported a renal transplant patient with a cavitary 4-cm, right-lower-lobe mass, again diagnosed by transbronchial biopsy; decreasing the patient's immunosuppressive therapy led to complete clearing of the lesion. Also in 1984, Crouch and coworkers[776] applied x-ray spectroscopic analysis to bilateral lower-lobe 0.5- to 2.5-cm nodules that mimicked metastatic carcinoma to the lung in an alcoholic patient.

Malakoplakia has been associated with *R. equi* in the lungs in AIDS cases.[774,777] Even when not described as characteristic malakoplakia, finely granular smaller macrophages filled with gram-positive coccobacillary, vividly PAS-positive organisms reminiscent of Whipple's and even *Mycobacterium avium-intracellulare* histiocytoses may be seen.[778]

Grossly, the soft yellow plaques, for which the disease in named, occur predominantly in hollow organs. In other organs, including the lung, these areas vary from gray-tan to yellow. Smaller nodules are more solid, but necrosis is frequent even in these, and may lead to cavitation. Some lesions extend across pulmonary fissures.[774]

Histologically the background architecture may be obliterated, but at the edges alveolar filling may be seen. Multiple granular-to-vacuolated von Hansemann's histiocytes are seen better at higher power (Fig. 8.77). These consist of histiocytes with distinct cytoplasmic borders and cytoplasmic granules that vary greatly in size. These granules stain positively with PAS, with and without diastase digestion; they are lipid rich on frozen sections. Round intracytoplasmic blue-gray target-like or owl's-eye–like Michaelis-Gutmann bodies are important findings in confirming this disorder (Fig. 8.77). They range from 3 to 20 μm in diameter, and may be round, oval, or slightly indented on one side. Most are intracellular but some may be extracellular. They tend to have a central or eccentric dark mass surrounded by a clear zone with peripheral accentuation and may stain with iron and calcium stains. Analysis by Crouch et al.[776] has shown some variation in the amounts of calcium, phosphorus, and iron among individual bodies examined. This variability has led this group to suggest that carbonates may be present along with organic phase lipids and polysaccharides. About 95% of the content of the bodies is organic.

Careful search may yield some histiocytes in which bacteria can be detected with Gram stain or acid-fast stains, but these are usually not numerous and are not seen in all cases. Electron microscopy indicates that many cells have assorted lysosomes, and some have identifiable bacteria in their cytoplasm. In at least one case the intracellular bacteria *(R. equi)* had thicker cell walls than cultured bacteria, possibly associated with intracellular persistence.[779] Similar discrepancies in ultrastructural appearance of intracellular bacteria (partially acid-fast) and cultured bacteria (non–acid-fast) have been described for *Legionella micdadei*.[501] Along with the characteristic histiocytic infiltration, plasma cells and lymphocytes are present. Multinucleate giant cells are absent or infrequent. Granulation tissue and fibrosis are common at the edges of the areas of inflammation, but they may be mixed with histiocytes.

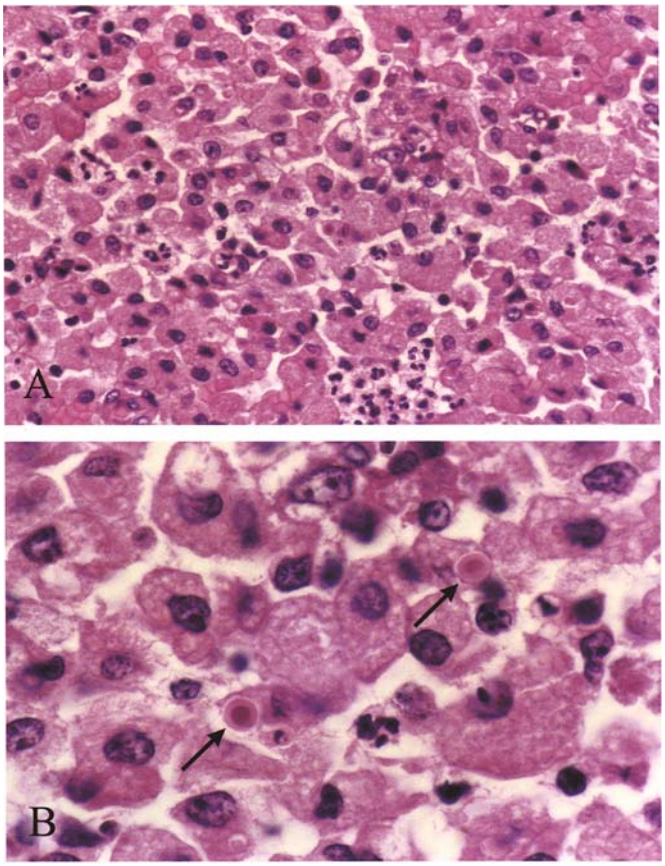

FIGURE 8.77. Malakoplakia due to *Rhodococcus equi.* **A.** Sheets of granular macrophages with pockets of neutrophils. **B.** Michaelis-Guttman bodies with central densities (arrows). (Courtesy of Dr. Carol Farver, Cleveland Clinic.)

Underlying diseases are common in malakoplakia and immunosuppression may play a role in the pathogenesis of the reaction, but many cases occur without these associations. Careful search for Michaelis–Gutmann bodies may be necessary, but once these are found, the pathologist will be rewarded with a more secure diagnosis. The diagnosis of malakoplakia should be preserved for those

cases with identifiable Michaelis-Gutman bodies. Clear, foamy, and granular macrophage diseases, which are in the differential diagnosis, include endogenous lipid pneumonia, Whipple's disease, *M. avium-intracellulare* infection, as well as Niemann-Pick, Gaucher's, and other storage diseases. None of these entities has been reported to contain Michaelis-Gutmann bodies.

Whipple's Disease

In 1907 G.H. Whipple[780] noted fatty deposits in intestines and mesenteric nodes of a patient with diarrhea, abdominal pain, weight loss, and arthralgia. He called the complex "intestinal lipodystrophy"; it has also been called "lipogranulomatosis." As these names do not adequately describe the variety of organs that can be involved or the true character of the process, most authors now refer to the disease simply as Whipple's disease. Whipple himself originally noted that the fat present was mostly intracellular and was probably secondary to chylous drainage obstruction. He also astutely noted some argyrophilic-staining bodies on Levaditi silver stain and suggested these might be bacteria.

A bacterial etiology was suspected for decades, but the etiologic agent proved elusive. In 1949 Black-Schaffer[781] noted vivid PAS staining in the typical Whipple's disease macrophages. When Whipple's original case was reexamined years later, PAS particles were observed in the macrophages.[782] In 1961 two groups of investigators determined that these bodies were most likely bacteria.[783,784] Elucidation of the bacterial etiology had to await the molecular age. Relman and colleagues[785] reported the presence of novel bacterial DNA in Whipple's lesions. The bacterium, which was subsequently named *Tropheryma whipplei*,[786] has now been cultivated in vitro,[787] although isolation is uncommon.

The clinical features of Whipple's disease have been reviewed by Maizel et al.[788] and by Durand and colleagues.[789] The gastrointestinal tract is the most common organ affected, but virtually any tissue may be involved, including the lung. Pulmonary involvement has been reviewed by Winberg and colleagues[790] and by Symmons et al.[791] Cough was noted in 50% of 98 cases reviewed by Enzinger and Helwig.[792] This symptom was attributed to nonspecific pleuritis, which was present in 72% of those autopsies reviewed in which the patients had a cough. Pleuropulmonary symptoms were reported in seven patients (13%) studied by Durand and colleagues.[789] Rarely pulmonary involvement is the initial manifestation of disease.[793]

Pericarditis was present at autopsy in 73% in the series of Enzinger and Helwig,[792] but was rarely diagnosed during life. Pleuripericarditis may precede gastrointestinal tract involvement by some time; in one report, the duration was 4 years.[794]

Typical organisms were described in the intima of pulmonary arteries in two patients who died of Whipple's disease.[795] Spain and Kliot[796] described classical Whipple's cells in pulmonary emboli in one case.

A carefully studied case, reported by Winberg et al.[790] is illustrated in Fig. 8.78. The patient had a progressively worsening cough for 2 years before he developed diarrhea for the first time. His chest radiograph showed bilateral peribronchiolar basilar infiltrates and a diffuse accentuation of an interstitial pattern. Transbronchial biopsy was not diagnostic, but an open lung biopsy showed the diagnostic lesions. A follow-up gastrointestinal biopsy confirmed Whipple's disease. In this case the affected macrophages formed nodules in the interstitium around bronchioles and, to a lesser degree, around blood vessels of all sizes (Fig. 8.78). Grossly, the nodules were described as firm and white, up to 5 mm in diameter. The typical PAS-positive enlarged histiocytic cells infiltrated both the bronchiolar smooth muscle and bronchiolar mucosa. Plasma cells and lymphocytes were scattered among these cells, but were limited in number. There were no giant cells and no evidence of necrosis or vasculitis. In this case, a large number of typical PAS-positive histiocytes was also noted in the pleural connective tissue, and it was proposed by these authors that pleural biopsy might have been used to make the diagnosis. Electron microscopy showed $0.2 \times 1.0 \times 1.5\,\mu m$ bacilliform structures.

The histochemical staining of *T. whipplei* produces fine pale to light-blue vacuolation when H&E is employed. The bacteria are brightly colored by PAS, with or without diastase digestion; histochemical stains that include PAS, such as Gridley's stain for fungi, also produce positive results. They are moderately to intensely Gomori methenamine silver (GMS) positive and are acid-fast negative. The bacteria may also be documented by enzyme immunochemistry is tissue sections.[797,798]

The differential diagnosis of Whipple's disease in the lung includes malakoplakia and, less often, *Pneumocystis* or *Legionella* infection; endogenous lipid pneumonia or other causes of xanthogranulomatous inflammation; and lipid storage disorders, such as Niemann-Pick and Gaucher's diseases. In these cases the alveoli are more commonly filled with distended macrophages, while the inflammation in Whipple's diseases is more commonly interstitial. A PAS stain may help to distinguish the organisms in Whipple's disease, but is not specific; immunohistochemistry provides a more specific diagnosis if culture is not possible. Care must be taken to exclude other infections, such as *Mycobacterium avium* complex, in which foamy to granular interstitial macrophages that contain PAS-positive organisms may be found. These bacteria are larger than those of Whipple's disease and are vividly acid-fast.

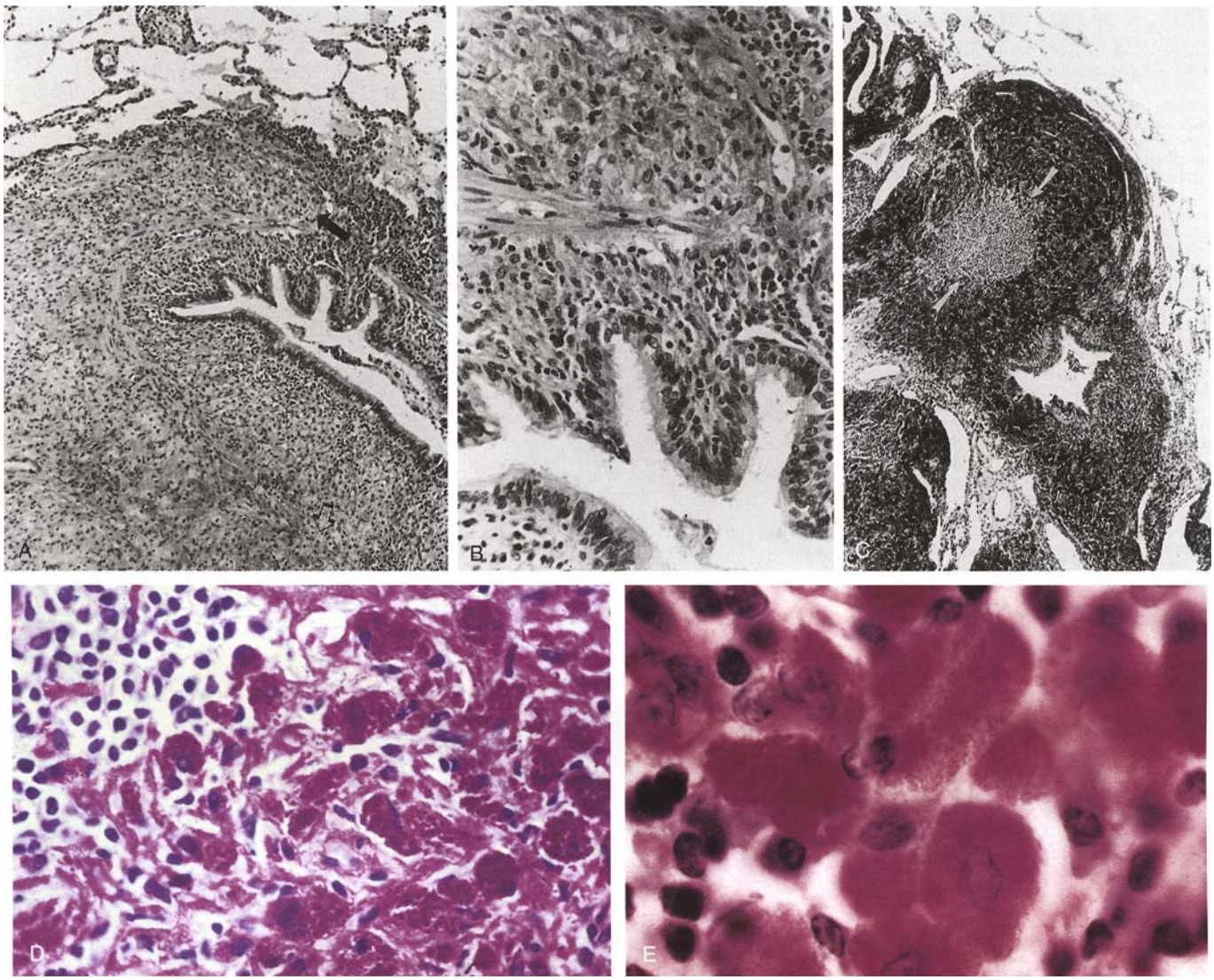

FIGURE 8.78. Whipple's disease. **A.** Reticulonodular infiltrates consist of predominantly interstitial collections of typical macrophages about the bronchioles, infiltrating the muscle coat (open arrow) at the bottom edge, with extension into the submucosa. The solid arrow is the site of a higher power view (B). **B.** Higher power shows granular macrophages in submucosa and outside muscularis. **C,D.** Periodic acid-Schiff (PAS) stain intensely stains bacteria. **C.** Area indicated by arrows is lymphoid nodule. (PAS.) **D.** Higher power view of transition from PAS-positive macrophages to unstained lymphoid nodule from **C.** (PAS.) (Courtesy of Dr. M.E. Rose and Dr. R.E. Horowitz, St. Joseph Medical Center, Burbank, CA, and Dr. C.D. Winberg, City of Hope National Medical Center, Duarte, CA.) E. Oil immersion of PAS stain shows how minute and delicate are the organisms.

Cat-Scratch Disease and Bacillary Angiomatosis

Cat-scratch disease is another historical infection for which an infectious etiology was long suspected, but only proven in recent years. The classic illness manifests as localized lymphadenopathy with characteristic necrotizing granulomas as the histologic response. The demonstration of bacteria in sections by silver impregnation techniques was followedH by the isolation of a bacterium, subsequently named *Afipia felis*, from infected tissue.[799] At about the same time a newly recognized bacterium was isolated from the blood of patients who were immunosuppressed by bone marrow transplantation or AIDS.[800] This organism, which was eventually classified as *Bartonella henselae*, was soon recognized as the predominant cause of cat-scratch disease, as well as a distinctive vascular proliferation, bacillary angiomatosis, that was seen in immunosuppressed

patients.[801–804] An additional species, *Bartonella quintana* (formerly *Rickettsia quintana*) was found to be the etiologic agent of similar infections, as well as classic trench fever.[805] With an etiologic agent in hand it was soon recognized that the clinical spectrum of cat-scratch disease was much broader than previously appreciated.

The diagnosis of *Bartonella* infection is usually made serologically, because of the difficulty in culturing the bacterium.[806,807] Association of a particular lesion, especially an unusual one, however, requires documentation of the putative etiologic agent in the tissue directly. Demonstration of bacillary forms with silver impregnation stains is suggestive, but not etiologically specific. An alternative is immunohistochemical or molecular documentation of the bacterium in the affected tissue.

Pulmonary infection with *Bartonella* spp. has been reported,[808] but is distinctly unusual. In several patients from whom material for histologic analysis was obtained the tissue reaction resembled the mixed inflammatory infiltrates of bacillary angiomatosis, with or without proliferation of small blood vessels, rather than the granulomas of cat-scratch disease.[809,810] Bacillary forms were demonstrated with silver impregnation stains; in one case masses of bacteria were seen as amorphous purplish masses in areas of necrosis when the sections were stained with H&E.[810] There is one report of asymptomatic interstitial infiltrates demonstrated in chest radiographs (and presumed to be caused by *Bartonella*) in an immunocompetent child with probable cat-scratch disease.[811]

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