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Microbiology

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Cytologists are likely to encounter infectious diseases either because a cytology sample was obtained for diagnostic purposes or incidentally when an infectious process or microorganism is discovered in the material they are reviewing. In order to render an accurate diagnosis, and correctly identify clinically important species or microorganisms, a good understanding and knowledge of microbiology is essential. This chapter provides a broad overview of microbiology that is relevant to the practicing cytologist, but is not intended to replace standard microbiology texts.

Viruses

- Viruses replicate only inside host cells. Their particles (called virions) consist of DNA or RNA and a capsid (coat) that may be surrounded by a lipid envelope. Once they attach to and penetrate cells, they uncoat and replicate so that their progeny may be released following host cell lysis.
- Viral infection may cause cell death, proliferation, or neoplastic transformation (oncogenesis) (Table 4.1). Tumor viruses may promote cancer by expression of viral oncoproteins (or oncogenes) and/or inactivation of tumor suppressor genes.

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TABLE 4.1. Viral induced tumors.

Virus	Tumor
Epstein-Barr virus (EBV)	Non-Hodgkin lymphoma (e.g., Burkitt lymphoma, post-transplant lymphoproliferative disorder, plasmablastic lymphoma) Hodgkin lymphoma Carcinoma (e.g., nasopharyngeal carcinoma, gastric carcinoma) Smooth muscle tumor Follicular dendritic cell sarcoma
Kaposi sarcoma herpesvirus/ human herpesvirus-8 (KSHV/HHV8)	Kaposi sarcoma Non-Hodgkin lymphoma (e.g., primary effusion lymphoma) Castleman disease
Human papillomavirus (HPV)	Anogenital dysplasia and carcinoma Oropharyngeal dysplasia and carcinoma
Hepatitis viruses (HBV, HCV)	Hepatocellular carcinoma
Human T-cell lymphotropic virus type 1 (HTLV-1)	Adult T-cell leukemia/lymphoma
Merkel cell polyomavirus (MCPyV)	Merkel cell carcinoma

- In general, viruses are too small to be identified directly by light microscopy. Viruses that remain latent often do not cause apparent changes to infected cells. However, several viruses may cause cytopathic changes (Fig. 4.1) that affect the nucleus (e.g., inclusions, margination, multinucleation), cytoplasm (e.g., koilocytosis, syncytial giant cell formation), and/or entire cell (e.g., cytomegaly, ciliacytopthoria). Recognition of these changes can be life-saving as this would initiate confirmatory studies and/or therapy (Fig. 4.2).
- Superinfection by pyogenic bacteria is a complication of many viral infections that may mask subtle viral changes.

Papillomaviruses

- Papillomaviruses (genus) are nonenveloped viruses that contain double-stranded circular DNA molecules that replicate exclusively in skin and/or mucosal keratinocytes. They belong to the *Papillomaviridae* family.

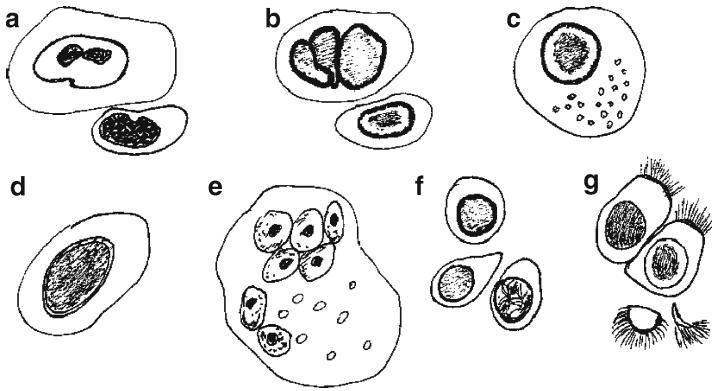


FIG. 4.1. Viral cytopathic changes. (**a**) HPV showing a large binucleate koilocyte and adjacent smaller high grade squamous intraepithelial lesion (HSIL) cell. (**b**) Herpes simplex virus showing a large multinucleated epithelial cell with cowdry A inclusions and a smaller cell with an intranuclear cowdry B inclusion. (**c**) CMV infected cell showing enlargement (cytomegaly), an intranuclear inclusion ("owl's-eye" appearance), and intracytoplasmic inclusions. (**d**) *Molluscum contagiosum* infection showing a keratinocyte with an intranuclear inclusion (molluscum body). (**e**) Measles (or RSV) infected syncytial giant cell with intranuclear inclusions. (**f**) BK polyomavirus infected epithelial cells (decoy cells) showing early (ground glass) and late ("fish-net stocking") intranuclear inclusions, as well as a comet cell in the middle with eccentric cytoplasm. (**g**) Adenovirus infected pneumocytes showing "smudge cells" with inclusions filling the nucleus and decapitated ciliated cells (ciliocytophthoria).

- Their genome is divided into an early (E) region that encodes genes E1–E7, and a late region (L) that encodes the capsid genes L1 and L2. In oncogenic human papillomavirus (HPV) types, the genes E6 (binds to p53) and E7 (binds to retinoblastoma [Rb] protein) are key players in transforming cells.
- HPV infection causes various diseases including warts (verruca), anogenital lesions (condylomata acuminata, intraepithelial neoplasia) and cancer, epidermodysplasia verruciformis (genodermatosis), oral and laryngeal papillomas, as well as orpharyngeal and conjunctival cancer. Genital HPV infection is covered in greater detail in Chap. 5.

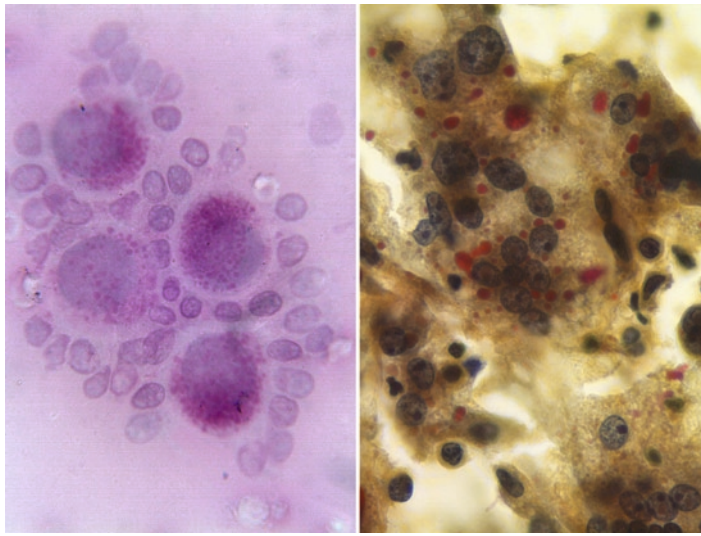


FIG. 4.2. Viral cytoplasmic inclusions. (*Left*) Cytomegalovirus infected cells (MGG stain, high magnification). (*Right*) Measles infected cells (Phloxine tartrazine stain, high magnification) (image courtesy of Dr. Pawel Schubert, University of Stellenbosch, Cape Town).

Herpesviruses

- Herpesvirues (family Herpesviridae) are DNA viruses that may cause latent or lytic infections. Reactivation of latent viruses has been implicated in a number of diseases.
- A major hallmark of herpes infection is the ability to infect mainly epithelial mucosal cells and/or lymphocytes. Cytomegalovirus (CMV) can infect many cells types including epithelial cells, endothelial cells, neuronal cells, smooth muscle cells, and monocytes.
- There are eight types of herpesvirus that may infect humans (Table 4.2). They include the Alphaherpesviruses (HSV and Varicella-Zoster virus [VZV]), Betaherpesviruses (CMV, HHV6, HHV7), and Gammaherpesviruses (Epstein-Barr virus [EBV] and KSHV). EBV and KSHV are oncogenic.
- *Herpes simplex virus types 1 and 2* (*HSV-1 and HSV-2*) have similar characteristics. Viral infection typically results in the

TABLE 4.2. Human herpesviruses (HHV).

HHV type	Virus name	Target cells	Disease
HHV1	Herpes simplex virus type 1 (HSV-1)	Mucoepithelium	Oral and/or genital herpes
HHV2	Herpes simplex virus type 2 (HSV-2)	Mucoepithelium	Oral and/or genital herpes
HHV3	Varicella-Zoster virus (VZV)	Mucoepithelium	Chickenpox Shingles
HHV4	Epstein-Barr virus (EBV)	Lymphocytes and epithelium	Infectious mononucleosis Non-Hodgkin lymphoma Hodgkin lymphoma Nasopharyngeal carcinoma Lymphomatoid granulomatosis Gastric carcinoma Oral hairy leukoplakia
HHV5	Cytomegalovirus (CMV)	Epithelium, monocytes, lymphocytes	Acute (mono-like) illness Systemic illness (e.g., pneumonia, hepatitis) Retinitis
HHH6	Roseolovirus	T lymphocytes and others	Sixth disease (roseola infantum or exanthem subitum)
HHV7	Human herpes virus-7 (HHV-7)	T lymphocytes and others	Sixth disease (roseola infantum or exanthem subitum)
HHV8	Kaposi's sarcoma-associated herpes virus (KSHV)	Lymphocytes and endothelium	Kaposi sarcoma Non-Hodgkin lymphoma Multicentric Castleman disease

formation of Cowdry type A and B inclusions. Both types of HSV can infect oral (e.g., cold sores) or genital mucosa. HSV-2 is normally spread sexually, which is why genital herpes is usually the result of HSV-2 infection. Infection may also cause herpes keratitis, gladiatorum (skin lesions), visceral and CNS infection, and neonatal herpes.

- *VZV* typically causes chickenpox (Varicella) in childhood and shingles (Zoster) in adults, which is usually severe in patients with acquired immunodeficiency syndrome (AIDS). Approximately 15% of patients may develop pneumonia. Infection in utero can cause congenital varicella syndrome. The cytopathic effect of VZV infection is similar to that seen with HSV.
- *EBV* has a tropism for epithelial cells (e.g., oral and nasopharynx) and B lymphocytes, binding to its receptor (CD21). Primary infection may cause infectious mononucleosis. Infected B-cells activate T-cells, the cause of atypical lymphocytosis in infectious mononucleosis. Latent infection in cells is characterized by the expression of latent membrane proteins (LMP) 1 and 2, EBV nuclear antigens (EBNAs), and EBV-encoded RNAs [EBERs]. EBV-associated malignancies are associated with latent gene expression (Table 4.3).
- *CMV* is the largest virus to infect humans. It produces cytomegalic cells with characteristic "owl's eye" nuclear inclusions. Most infections are asymptomatic. However, in immunosuppressed persons CMV can be a major problem. CMV pneumonia is the most common life-threatening complication after transplantation.
- *Human herpes virus 6 and 7* (*HHV6 and HHV7*) both infect T cells. They cause sixth disease typically in children, where a transient skin rash on the trunk and neck follows an episode of fever.
- *Kaposi's sarcoma-associated herpes virus/Human herpesvirus-8* (*KSHV/HHV8*) was the eighth herpes virus to be discovered. Most viral genes are expressed in lytic infection. The five genes expressed in latent infection are key to oncogenesis, which includes the latency-associated nuclear antigen (LANA). The immunohistochemical stain LNA-1 targets LANA within the nuclei of KSHV infected cells. KSHV has an etiologic role in Kaposi sarcoma, certain lymphomas like primary effusion lymphoma (PEL), and multicentric Castleman disease.

TABLE 4.3. Patterns of latent gene expression in EBV.

		Latency I	Latency II		Latency III	
EBV gene	Acute infection	Burkitt lymphoma	Hodgkin lymphoma	Nasopharyngeal carcinoma	PCNSL	PTLD
EBNA1	+	+	+	+	+	+
EBNA2	+	−	−	−	+	+
EBNA3	+	−	−	−	+	+
LMP1	+	−	+	+	+	+
LMP2	+	−	+	+	+	+
EBER	+	+	+	+	+	+

PCNSL primary central nervous system lymphoma; *PTLD* post-transplant lymphoproliferative disorder

Respiratory Viruses

- *Influenza* and *Parainfluenza viruses* can cause severe respiratory tract disease (e.g., pneumonia, bronchitis, and bronchiolitis). As infection usually does not cause characteristic cytologic findings, the diagnosis requires isolation and identification of the virus in the laboratory or a rise in serum antibodies.
- *Coronavirus* causes illness ranging from the common cold to severe acute respiratory syndrome (SARS). Respiratory samples may show atypical reactive pneumocytes with or without background inflammation and marked fibrin exudate in cases with diffuse alveolar damage (DAD).
- *Respiratory syncytial virus* (*RSV*) causes lower respiratory tract infections mainly in childhood. RSV belongs to the same Paramyxoviridae family as measles (Rubeola) and mumps viruses. Both RSV and measles pneumonia can cause multinucleated syncytial giant cells containing intranuclear and inconspicuous usually paranuclear cytoplasmic inclusions. Multinucleated giant cells are usually rare, but when identified may contain up to 35 nuclei.
- *Adenoviruses.* They were named after being first isolated from adenoid samples. There are 55 described serotypes in humans that cause respiratory tract infections (e.g., pharyngitis, pneumonia). Infection may also cause gastroenteritis, conjunctivitis, hemorrhagic cystitis, meningoencephalitis, hepatitis, and disseminated disease. Early infected cells may display small eosinophilic inclusions. With late infection, basophilic intranuclear inclusions eventually obscure the nucleus producing a characteristic "smudge cell."

Polyomaviruses

- Most people are infected with these viruses and hence are seropositive for polyomaviruses. These double-stranded DNA viruses tend to only cause infection in immunosuppressed individuals, and are all potentially oncogenic. They fall under the SV40 (Simian vacuolating virus 40) clade seen in monkeys, except for Merkel cell polyomavirus.
- *BK virus* (BKV) has a tropism for cells of the genitourinary tract. BKV may cause nephropathy in 1–10% of renal transplant

patients resulting in the loss of their renal allograft. Reactivation in the kidneys and urinary tract results in shedding of infected cells, virions, and/or viral proteins in the urine. Infection can thus be diagnosed using urine cytology looking for cells with polyomavirus inclusions of the nucleus, as well as PCR. BKV may also cause ureteral stenosis in renal transplant patients and asymptomatic hemorrhagic cystitis, usually after bone marrow transplantation.

- *JC virus* (*JCV*) may infect the respiratory tract, kidneys, or brain. CNS infection can cause fatal progressive multifocal leukoencephalopathy (PML) in AIDS patients.
- *Merkel cell polyomavirus* (*MCV or MCPyV*). This recently discovered virus (in 2008) causes around 80% of Merkel cell carcinomas. Although lymphocytes may serve as a tissue reservoir for MCV infection, only rare (approximately 2%) of hematolymphoid malignancies show evidence for MCPyV infection by DNA PCR.

Poxviruses

- *Molluscum contagiosum virus.* Infection involves the skin and occasionally the mucous membranes. There are four types of MCV (MCV-1–4). Skin lesions are self-limited and pearly in appearance with an umbilicated (dimpled) center. Infected cells are characterized by molluscum bodies (also called Henderson-Paterson bodies). Unlike herpes, this virus does not remain latent. As patients do not develop permanent immunity, repeated infections can occur.

Retroviruses

- Retroviruses are enveloped viruses that belong to the viral family *Retroviridae.* They are RNA viruses that replicate in host cells using the enzyme reverse transcriptase to produce DNA from its RNA genome. DNA is then incorporated into the host genome.
- *Human immunodeficiency virus* (*HIV*), *types 1 and 2*. HIV belongs to the retrovirus family. Infection causes AIDS. Details are covered in greater detail in Chap. 13.
- *Human T-cell lymphotrophic virus* (*HTLV*), *types 1 and 2.* HTLV-1 is the first recognized retrovirus that causes adult T-cell

leukemia/lymphoma (ATLL). Infection may also be involved in certain demyelinating diseases. Infected lymphocytes in the peripheral blood produce characteristic "flower cells."

Miscellaneous Viruses

- *Hepatitis viruses*. Several viruses may cause hepatitis including Hepatitis A (RNA picornavirus), Hepatitis B (DNA hepadnavirus), Hepatitis C (RNA flavivirus), Hepatitis E (RNA calicivirus), and Hepatitis D (Delta agent). They usually do not cause viral cytopathic changes seen in cytology samples. However, in liver tissue chronic hepatitis B virus (HBV) can cause a ground-glass appearance of hepatocytes due to the accumulation of HBsAg within the endoplasmic reticulum. Chronic infection with HBV and hepatitis C (HCV) may lead to cirrhosis, liver dysplasia, and ultimately hepatocellular carcinoma.
- *Parvoviruses.* These are among the smallest known DNA viruses. Parvovirus B19 (B19V) causes fifth disease (erythema infectiosum) and arthropathy. Infection of erythroid precursors in the bone marrow may cause severe anemia characterized by giant normoblasts and intranuclear inclusions with a ground glass appearance that tend to compress the chromatin against the nuclear membrane. Cells with parvovirus B19 inclusions have been reported in cytology fluid specimens from fetal cases with hydrops fetalis.

Bacteria

- Bacteria (singular: bacterium) are single-celled microorganisms that measure 0.5–5.0 μm in length. Mycoplasma spp. are among the smallest bacteria. Bacteria have a wide range of shapes. Most are spherical (cocci) or rod-shaped (bacilli), but they may also be curved or spiral-shaped (e.g., spirochaetes, *Helicobacter pylori*). Some bacteria are described as being coccobacilli because they have the ability to exist as a coccus, bacillus, or intermediate form (e.g., *Haemophilus influenzae*, *Rhodococcus equi*, *Bartonella* spp.). Bacteria may also form pairs (e.g., diploids), chains (e.g., Streptococcus), or clusters (e.g., Staphylococcus). Some bacteria may also have flagella.

- Anerobic bacteria do not need oxygen for growth. Some anerobes die when oxygen is present (obligate anerobes), whereas others will utilize oxygen if it is present (facultative anaerobes). They are found in normal flora (e.g., *Fusobacterium* in the mouth, *Bacteroides fragilis* in the large bowel, *Lactobacillis* in the vagina). These bacteria can usually be isolated from abscesses, aspiration pneumonia, empyema, and wounds. Material being collected from sites that do not harbor indigenous flora (e.g., body fluids other than urine and fine needle aspirates) should always be cultured for anerobic bacteria.
- Bacteria, along with some fungi (mainly *Candida* spp.) and archaea (single-celled microorganisms), make up the normal human flora of the skin, mouth, gastrointestinal tract, conjunctiva, and vagina (lactobacilli). Loss of normal flora may permit the unfavorable growth of harmful pathogens that can lead to infection.
- Some bacteria form biofilms, which are bacterial aggregates embedded within a self-produced matrix (slime). These bacterial clusters, seen associated with amorphous mucoid material, may be encountered in cytology specimens related to catheter infections, *Pseudomonas aeruginosa* pulmonary infections in cystic fibrosis, middle ear infections, joint prostheses, and dental (gingival) disease.

Gram-Positive and Gram-Negative Bacteria

- Bacteria can generally be divided into Gram-positive and Gram-negative bacteria on the basis of their reaction to the Gram stain. Most bacteria can be classified into one of the following four groups: Gram-positive cocci, Gram-positive bacilli, Gram-negative cocci, and Gram-negative bacilli.
- *Gram-positive bacteria* stain dark blue (violet) by Gram staining because they retain the crystal violet stain as a result of the abundant peptidoglycan in their cell wall. Gram-positive cell walls typically lack the outer membrane found in Gram-negative bacteria.
- *Gram-negative bacteria* cannot retain the crystal violet stain. Hence, they take up the counterstain (safranin or basic fuchsin) instead and with Gram staining appear red or pink (Figs. 4.3 and 4.4).

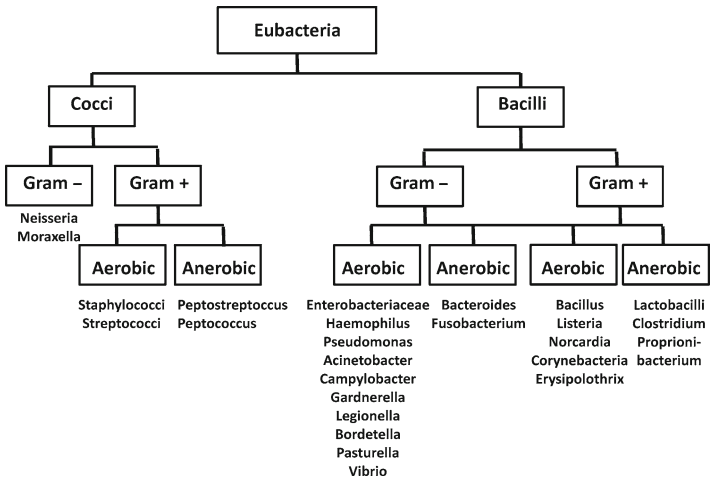


FIG. 4.3. Bacteria divided according to their shape (cocci or bacilli), Gram staining properties, and dependence upon oxygen for growth.

Mycobacteria

- Mycobacteria are aerobic Gram-positive rod-shaped bacilli that are acid–alcohol fast (so-called AFB) with acid fast stains (Fite, Ziehl-Neelsen, Kinyoun, and auramine rhodamine stains). *Mycobacterium tuberculosis* are strongly acid fast positive (stain deep red), thin, and slightly curved bacilli that measure 0.3–0.6 × 1–4 nm (Fig. 4.5). The bacteria of MAI are typically short and cocobacillary like. Beading may be seen in some mycobacteria, which represents nonuniform staining of the bacillus. For example, *M. kansasii* are characteristically long and broad and exhibit a cross-banded or barred appearance.
- Acid-fast staining of morphologically similar bacteria such as *Nocardia* and *Legionella* is a possible pitfall in the cytologic diagnosis of mycobacterial infection. Other organisms known to be acid-fast positive include micrococcus species, the oocysts of cryptosporidium species, *Isospora belli*, and sarcocystis.
- Mycobacteria are grouped on the basis of their appearance and rate of growth in culture (slow, intermediate, and rapidly growing). According to the Runyon classification there are three

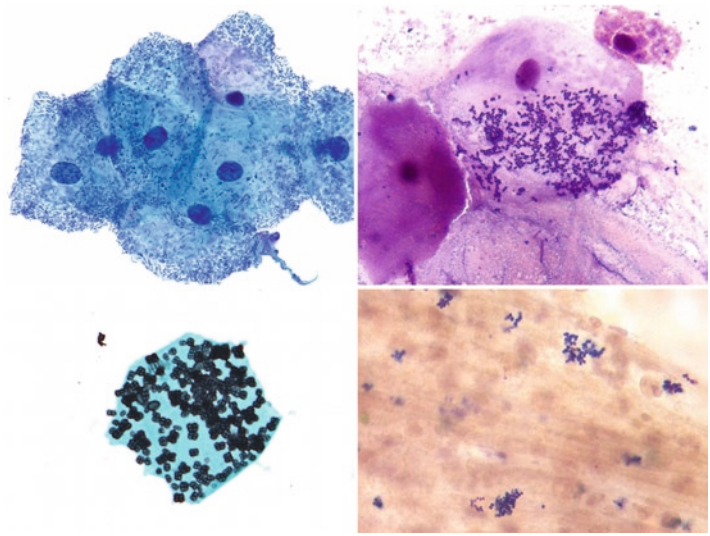


FIG. 4.4. Commonly encountered bacteria. (*Top left*) Lactobacilli are shown in a Pap test associated with normal squamous cells. These were discovered by the German gynecologist Albert Döderlein in 1892. (*Bottom left*) Sarcina forms seen attached to an oral squamous cell in a BAL specimen, showing its characteristic appearance in tetrads (buckets of eight elements). This type of bacteria is frequently observed as a commensal flora to the mouth (GMS stain, high magnification). (*Top right*) Sputum showing Gram positive diplococci of *Streptococcus pneumoniae* (Gram stain, high magnification). (*Bottom right*) Lung abscess FNA showing clusters of Gram positive *Staphyloccus aureus* (Gram stain, high magnification).

slow growing groups (photochromogens that develop pigments with light exposure such as *M. kansasii* and *M. marinum*, scotochromogens which become pigmented in darkness such as *M. scrofulaceum*, and nonchromogens such as *M. avium complex* [MAC]). Rapid growers include *M. chelonae* and *M. fortuitum*.

- For diagnostic and treatment purposes they can be classified into three main groups:
  - *M. tuberculosis complex*. This includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti, BCG* and *M. canetti*. Infection causes tuberculosis. The response to Bacillus Calmette-Guerin (BCG)

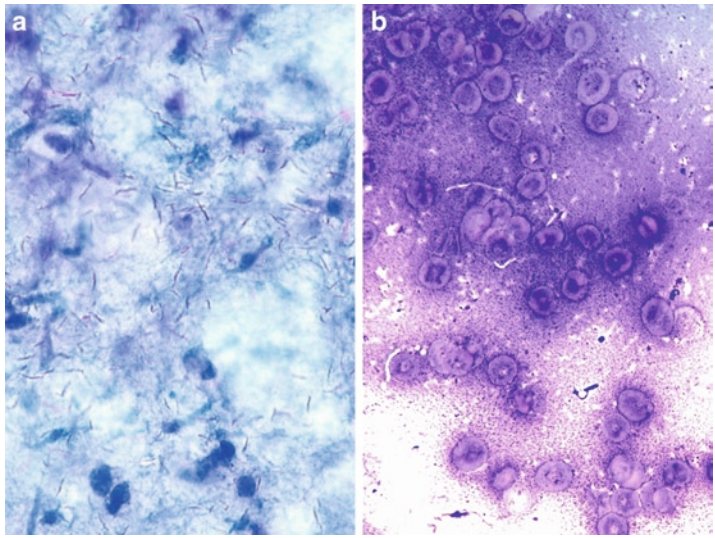


FIG. 4.5. *Mycobacterium tuberculosis* can be identified (**a**) with an acid stain (Ziehl-Neelsen stain, high magnification) or (**b**) negative staining of bacterial rods (Diff-Quik stain, high magnification).

vaccine in some infants and immunocomprised patients may cause postvaccinial disseminated infection presenting with lymphadenitis, osteomyelitis, and hepatic granulomas. BCG vaccine contains attenuated live bacilli of *M. bovis*.

- *Mycobacterium leprae* which causes leprosy (Hansen's disease).
- *Nontuberculous mycobacteria* (NTM), which include all of the other mycobacteria. These are also known as atypical mycobacteria, mycobacteria other than tuberculosis (MOTT), or environmental mycobacteria. Infections with these mycobacteria are increasingly being seen in immunosuppressed patients. Infection causes lung disease but may also disseminate to involve the hematopoietic system, gastrointestinal tract, as well as skin and soft tissue. While most NTM can be detected microscopically with an acid-fast stain, culture and/or molecular studies may be required to identify these species.

Filamentous Bacteria

- Bacteria can be elongated to form filaments (e.g., *Actinobacteria*, *Nocardia*, *Rhodococcus*, *Streptomyces*, *Actinomadura*). They can sometimes form complex, branched filaments that morphologically resemble fungal mycelia (mass of branching hyphae).
- These bacteria are usually part of the normal oral flora. Most infections are acquired by inhalation of the bacteria or via trauma.
- *Actinomyces* (genus) belong to the *Actinobacteria* (class of bacteria). Infection (actinomycosis) with these Gram-positive bacteria forms multiple abscesses and sinus tracts that may discharge sulfur granules. Actinomycosis is most frequently caused by *Actinomyces israelii*.
- *Nocardia* (genus) are weakly-staining Gram-positive bacteria that form partially acid-fast beaded branching filaments. There are a total of 85 species, although *Nocardia asteroides* is the species that most frequently causes infection (nocardiosis). Nocardial disease (norcardiosis) includes pneumonia, endocarditis, encephalitis, and/or brain abscess, as well cutaneous infections such as actinomycotic mycetoma (Figs. 4.6 and 4.7).

Chlamydia

- *Chlamydiae* are obligate intracellular Gram negative bacteria. They are classified taxonomically into a separate order (*Chlamydia*) because of their unique life cycle.
- Organisms occur in two forms: an elementary body (0.3 μm) that exists outside the host and infects host cells where it transforms into a reticulate body (0.6 μm). Following replication, new elementary bodies are released from the infected host cell when it ruptures.
- *Chlamydia* inclusion bodies may be identified within infected cells. However, the cytologic findings (e.g., in a Pap test) are not considered reliable. When stained with iodine, reticulate bodies can be visualized as intracytoplasmic inclusions. They can also

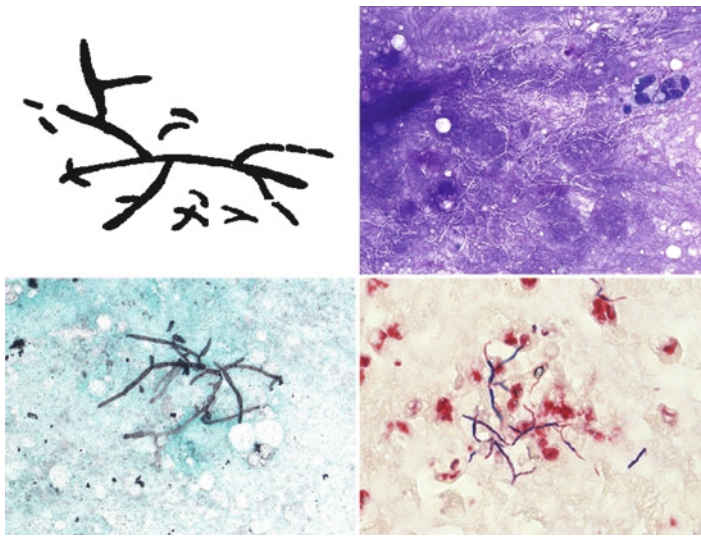


FIG. 4.6. *Nocardia.* (*Top left*) Diagrammatic illustration of branched filamentous bacteria. (*Top right*) Negative image of *Nocardia* in a direct smear from a brain abscess (Diff-Quik stain, high magnification). (*Bottom left*) *Nocardia* bacteria are shown highlighted with a GMS stain (high magnification). (*Bottom right*) Weakly Gram positive *Nocardia* (high magnification).

be stained with Giemsa or Gimenez methods as well as immunocytochemistry.

- Organisms are better detected by culture (gold standard) or other laboratory tests (e.g., enzyme immunoassay, leukocyte esterase test in urine, rapid *Chlamydia* test, and nucleic acid tests).
- Three species of *Chlamydia* are known to produce human disease:
  - *Chlamydia pneumonia* (also *Chlamydophila pneumoniae* and previously known as the TWAR agent). Infection causes pharyngitis, bronchitis, and atypical pneumonia. Less common infections include meningoencephalitis, arthritis, and myocarditis. An association with atherosclerosis and possibly lung cancer has been reported.

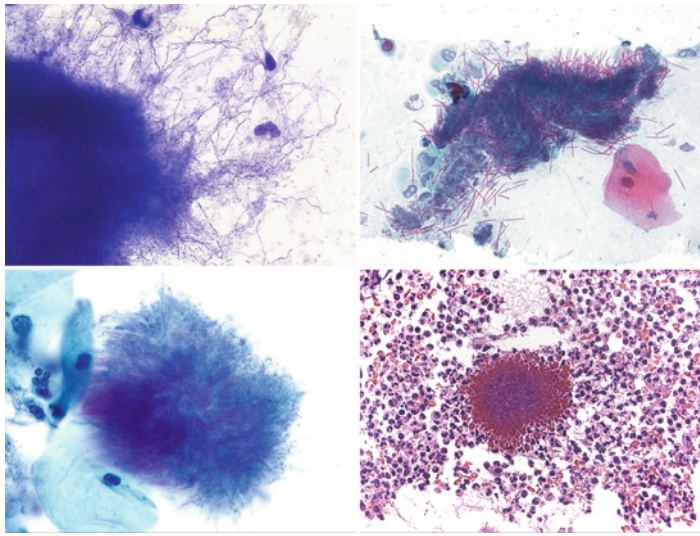


FIG. 4.7. *Actinomyces.* (*Top left*) Clump of long filamentous bacteria are shown (May-Grünwald-Giemsa stain, high magnification). (*Top right*) *Actinomyces* from the mouth contaminating a bronchoalveolar lavage ThinPrep specimen (Pap stain, high magnification). (*Bottom left*) Typical "dust bunny" seen on a cervical Pap test (Pap stain; high magnification). (*Bottom right*) Sulfur granule is shown in the center of the cell block preparation aspirated from an actinomycotic liver abscess (H&E stain, intermediate magnification).

- *Chlamydia trachomatis* (previously called TRIC agent). This includes three human biovars: trachoma (serovars A, B, Ba or C), urethritis (serovars D-K), and lymphogranuloma venereum (LGV, serovars L1, 2 and 3). Infection causes inclusion conjunctivitis (trachoma), pneumonia in neonates, and sexually transmitted disease in adults (e.g., cervicitis, urethritis, salpingitis, proctitis, epididymitis).
- *Chlamydia psittaci* (also called *Chlamydophila psittaci*) causes respiratory psittacosis and is acquired from birds (Fig. 4.8).

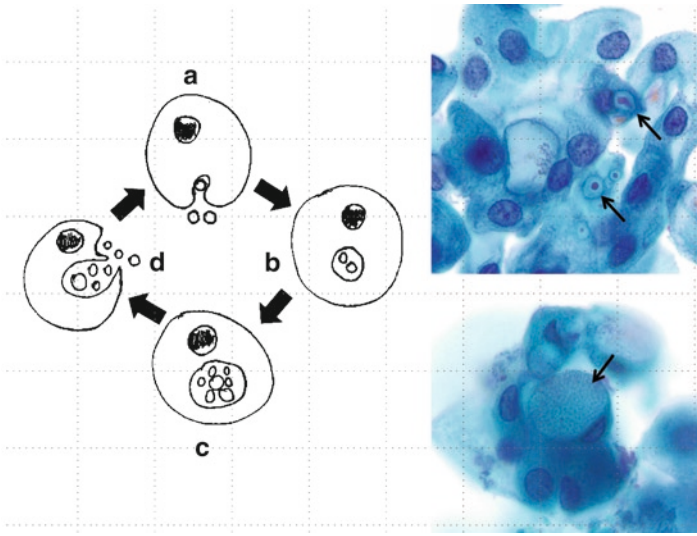


FIG. 4.8. Chlamydia. (*Left*) Chlamydia developmental cycle. Infectious elementary bodies that infect a host cell (**a**) transform into noninfectious reticulate bodies (**b**) which then multiply (**c**). Elementary bodies are then released following cell lysis that can infect new cells (**d**). *Chlamydia* inclusions containing (*top right*) elementary bodies (*arrows*) and (*bottom right*) reticulate bodies (*arrow*) are shown in squamous cells of a Pap test (Pap stain, high magnification).

Fungi

- On the basis of morphologic forms fungi can be divided into yeasts and hyphae.
- *Yeasts* are unicellular fungi. They reproduce by budding (forming *blastoconidia*) or fission. The term "yeast" is used only to describe a morphological form of a fungus and is of no taxonomic significance.
- *Hyphae* (single hypha) are multicellular fungi. Morphologically they are branching, thread-like tubular structures. Hyphae may lack cross walls (coenocytic or aseptate) or have cross walls (septate). A *mold* is a mass of hyphal elements (also called *mycelium*).

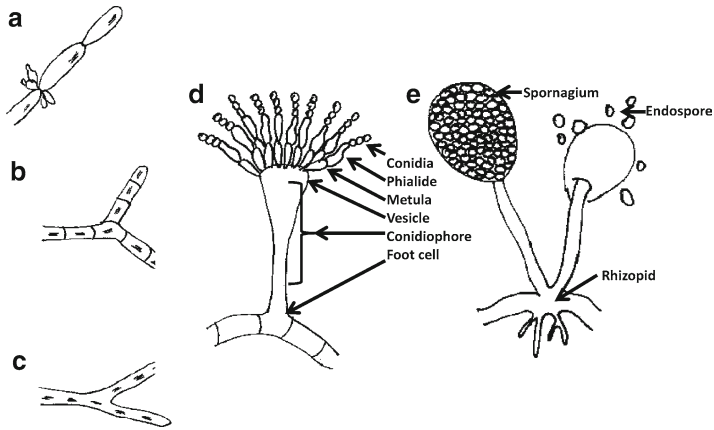


FIG. 4.9. Fungal morphology. Hyphae may be characterized as (**a**) pseudohyphae (e.g., *Candida* spp.), (**b**) septate (e.g., *Aspergillus*) or (**c**) coenocytic (aseptate) hyphae (e.g., Zygomycetes). Conidia (spores) develop from asexual fruiting structures such as (**d**) a conidiophore or (**e**) enclosed in a sac called a sporangium, in which case they are then called endospores.

- Hyphae can produce *conidia* (synonymous with *spores*). Large complex conidia are called macroconidia. Smaller more simple conidia are termed microconidia. When these conidia are enclosed in a sac (the *sporangium*) they are called *endospores*. A sporangium-bearing hypha is referred to as a sporangiophore.
- Dimorphism (*dimorphic fungi*) is the condition whereby a fungus can exhibit either the yeast form or the hyphal form, depending on growth conditions (Fig. 4.9).

Candida

- *Candida* is a polymorphic fungus that undergoes a yeast-to-mycelial transition. In clinical specimens, they produce pseudohyphae (hyphae that show distinct points of constriction resembling sausage links), rarely true septate hyphae, and budding yeast forms (blastoconidia).
- The yeast-like forms (blastoconidia) are oval and measure 3–5 μm in diameter

- Although typically seen extracellularly, intracellular *Candida* can mimic other small fungi such as *Histoplasma*. *Candida* usually exhibit variably sized yeast cells, lack a pseudocapsule, and elicit more of a suppurative reaction than a granulomatous response.
- *Candida* yeasts form part of the normal flora on the skin and mucous membranes of the respiratory, gastrointestinal, and female genital tracts. They often contaminate cytology samples from these sites. They may also colonize tissue (e.g., after prolonged antibiotic use, prolonged skin moisture, and in patients with diabetes).
- Infection may result from overgrowth or when introduced into the body (e.g., intravenously). Superficial infections include oropharyngeal and vulvovaginal candidiasis (thrush). Candidiasis may also become a systemic illness causing widespread abscesses, endocarditis, thrombophlebitis, endocarditis, eye infections, or involve other organs.
- *Candida albicans* is clinically the most significant member of this genus. *Candida glabrata* (previously known as *Torulopsis glabrata*) is a nondimorphic species (only has a yeast form) (Fig. 4.10).

Cryptococcus

- Cryptococci are small (5–15 μm) pleomorphic (ovoid to spheroid) yeasts that are characterized by often having a thick gelatin-like capsule and demonstrating narrow-based (teardrop-shaped) budding. They have thin walls and are occasionally refractile. Their capsules may have a diameter of up to five times that of the fungal cell, and form a halo on Diff-Quik, Pap, and India ink stains.
- Smaller (2–5 μm) capsule-deficient cryptococci can resemble other organisms with similar microforms (e.g., *Histoplasma*, *Candida*, and immature spherules of *Coccidioides immitis*). In such cases, with careful examination some weakly encapsulated yeasts can still be detected. Loss of capsular material usually elicits an intense inflammatory reaction characterized by suppuration and granulomas.
- Yeasts usually produce single buds, but multiple buds and even chains of budding cells may rarely be present.

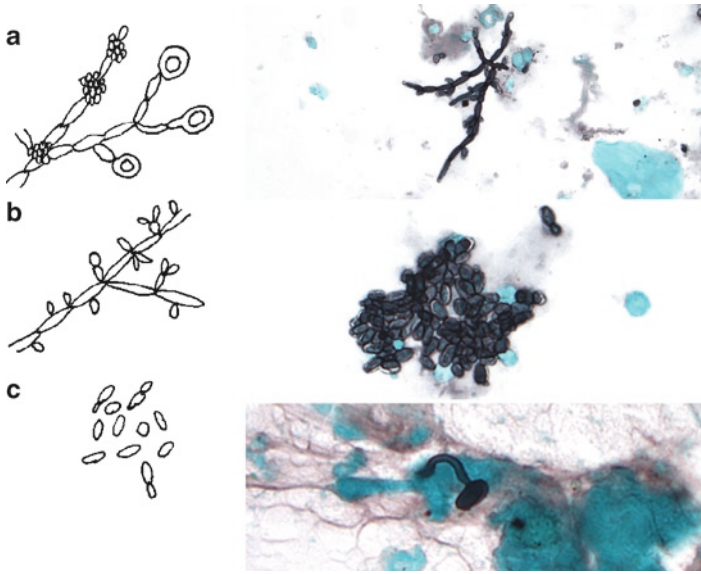


FIG. 4.10. *Candida* morphology. Pseudohyphae and yeast are shown of (**a**) *Candida albicans* and (**b**) *C. tropicalis*. (**c**) *C. glabrata* (*torulopsis*) only has a yeast form. The images of *Candida* on the *right* show classic examples of (*top*) pseudohyphae with distinct points of constriction along the fungal filaments, (*middle*) oval yeasts with a separate budding form present in the top right field of the image, and (*bottom*) a germ tube (germinating outgrowth) (GMS stains, high magnification).

- The presence of pseudohyphae-like elements and germ tube-like structures may be detected in some cases, mimicking *Candida*. However, this is rare and reported to be observed in older lesions of cryptococcosis where aberrant forms are frequently seen.
- Infection (cryptococcosis) arises mainly in immunosuppressed patients and may cause very little inflammation.
- *Cryptococcus neoformans* causes most infections, such as meningitis and meningoencephalitis in HIV positive patients.
- *Cryptococcus gattii* (formerly *Cryptococcus neoformans var gattii*), endemic in tropical areas of Africa and Australia, may cause cryptococcosis in immunocompetent individuals (Fig. 4.11).

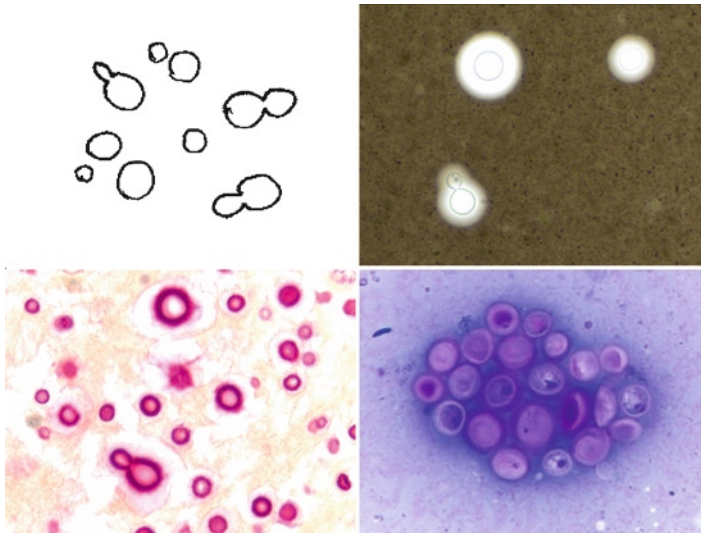


FIG. 4.11. *Cryptococcus*. (*Top left*) Diagram showing the pleomorphic yeast-like cells of *C. neoformans*, which have thin walls and exhibit narrow based budding forming teardrop structures. (*Top right*) India ink preparation of a CSF specimen from a patient with AIDS associated cryptococcal meningitis demonstrates unstained halos around the microorganisms due to their thick capsules (high magnification). (*Bottom left*) A mucicarmine stain demonstrates the mucinous capsules surrounding cryptococci (high magnification). (*Bottom right*) Cryptococci are shown with ovoid and cup-shaped forms that may resemble *Pneumocystis* organisms (Diff-Quik stain, high magnification).

Aspergillus

- *Aspergillus* genus consists of many mold species. Pathogenic species include *Aspergillus fumigatus* and *Aspergillus flavus*.
- These fungi consist of septate hyphae that branch at 45° angles. Other dichotomous hyphae that may mimic *Aspergillus* include the hyalinohyphomyces (e.g., *Fusarium*, *Penicillium*) and dermatophytes.
- Species specific conidiophores called fruiting bodies have swollen vesicles lined by phialides that give rise to many conidia. The presence of fruiting bodies in cytology samples are usually only seen in samples obtained from cavities or other well oxygenated areas.

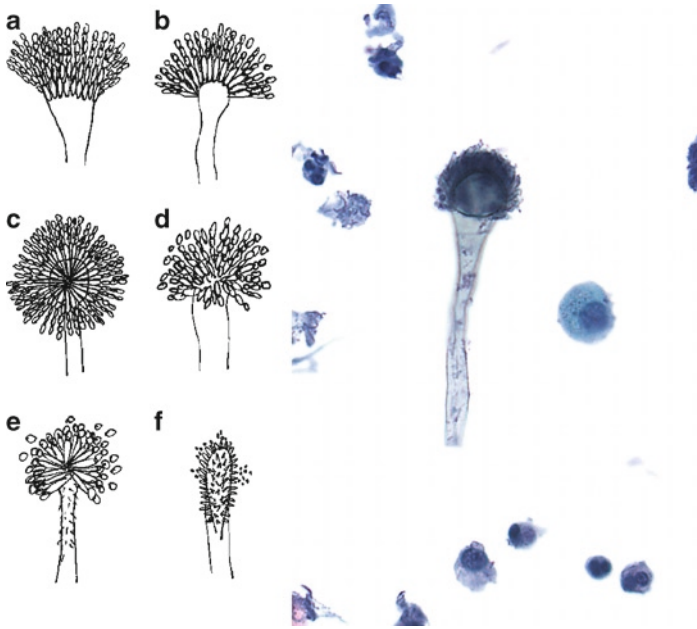


FIG. 4.12. Fruiting bodies of *Aspergillus* spp. (*Left*). Illustrations of the varied microscopic morphology of common *Aspergillus* spp. including (**a**) *A. nidulans* and *A. terreus*, (**b**) *A. fumigatus*, (**c**) *A. niger*, (**d**) *A. glaucus* group, (**e**) *A. flavus*, and (**f**) *A. clavatus*. (*Right*) Fruiting body of *A. fumigatus* is shown in a ThinPrep BAL specimen from a patient with a cavitary lung lesion (Pap stain, high magnification).

- Diseases caused by *Aspergillus* spp. (aspergillosis) include sinusitis, allergic bronchopulmonary aspergillosis, aspergilloma ("fungus ball") within lung cavities, and invasive disseminated aspergillosis (Figs. 4.12 and 4.13).

Zygomycetes

- The zygomycetes belong to the phylum Zygomycota (Table 4.4). The two orders that contain fungi causing human disease are the Mucorales and Entomophthorales. Most illness is linked to Rhizopus spp. of the Mucorales.

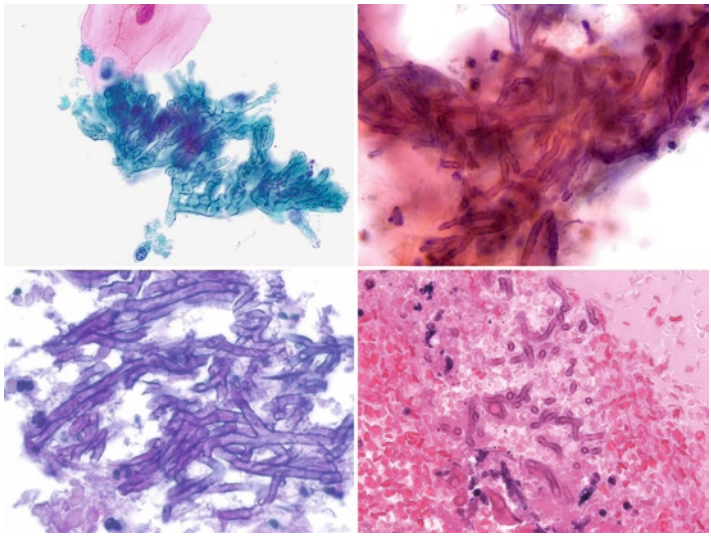


FIG. 4.13. *Aspergillus* hyphae with branching at 45° angles are shown (*top left*) in a ThinPrep specimen (Pap stain, high magnification), (*top right*) direct smear (Pap stain, high magnification), (*bottom left*) with a PAS stain (high magnification), and (*bottom right*) in a cell block (H&E stain, high magnification).

TABLE 4.4. Zygomycetes taxonomy.

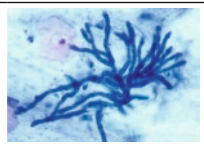
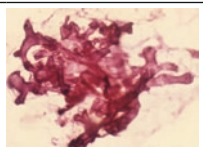
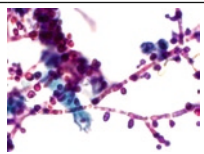
Phylum	Zygomycota		
Class	Zygomycetes		
Order	Mucorales		Entomophthorales
Family	Mucoraceae	Cunninghamellaceae Mortierellaceae Saksenaceae Syncephalastraceae Thamnidaceae	Ancylistaceae Basidioboaceae
Genus	Absidia Apophysomyces Mucor Rhizomucor Rhizopus		

- Zygomycetes are fungi characterized by the formation of spores (zygospores) and a vegetative mycelium. They have broad, ribbon-like, aseptate hyaline hyphae (coenocytic hyphae) with wide-angle branching. These morphological features are helpful in differentiating the zygomycetes from other fungal agents of infection that may be seen in cytologic specimens (Table 4.5).
- In cytology samples hyphal forms may be twisted, collapsed, or wrinkled making them hard to evaluate. Moreover, in tissue sections from biopsies or cell block material, folds and creases in the section may cause the hyphae to appear as if they have septae.
- In respiratory samples, the zygomycetes can be distinguished from dimorphic fungi and yeasts as they do not produce a yeast phase in this anatomic site.
- Samples are often associated with extensive necrosis and inflammation.
- Their isolation in the clinical laboratory reflects either environmental contamination or clinical disease (zygomycosis). Human zygomycosis is usually an opportunistic infection in immunocompromised hosts such as patients with diabetes mellitus, neutropenia, or using immunosuppressive therapy.
- Infection is associated with angioinvasive disease causing thrombosis, tissue infarction, and subsequent dissemination.
- Disease manifestations include rhinocerebral and pulmonary disease, and infrequently cutaneous, gastrointestinal, and allergic diseases (Fig. 4.14).

Dimorphic Fungi

- Dimorphic fungi can exist both as a mold form that consists of hyphae (when grown at room temperature outside the host) and as yeast (when grown at body temperature in the host). Therefore, in clinical samples obtained from patients the cytologist will encounter yeasts from these organisms (Table 4.6). Several such fungal species are potential pathogens.
- *Blastomyces*. The yeasts are 8–15 μm in size, have a double-contour refractile wall, and demonstrate broad-based budding. The most well-known species of this genus is *Blastomyces dermatitidis*, endemic to the United States (especially the southeastern, south central, and midwestern states) and Canada.

TABLE 4.5. Comparison between zygomycetes, *Aspergillus* spp., and *Candida* spp.

Morphologic feature	*Aspergillus*	Zygomycetes	*Candida*
Pap stain appearance			
Hyphal type	Septate	Aseptate	Pseudohyphae
Hyphal width	Consistently thin (2–3 μm wide)	Variable and wide (6–16 μm wide)	Consistently thin (2–3 μm wide)
Branching	45° angles	90° angles	Variable angles
Blastoconidia	Absent	Absent	Present
Sporulation	Present with air exposure	Absent	Absent

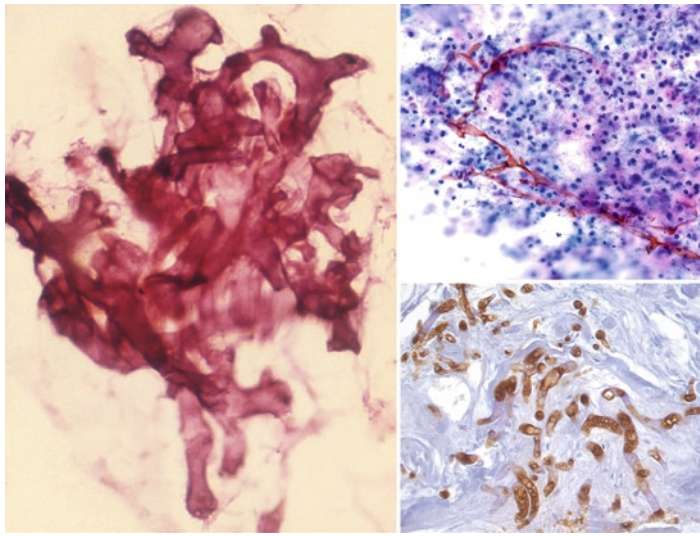


FIG. 4.14. Zygomycetes. (*Left and top right*) Zygomycete hyphae are shown characterized by broad, aseptate (coenocytic) hyphae that display wide-angle branching (Pap stain, *left* high magnification, *top right* intermediate magnification). (*Bottom right*) Fungal hyphae are shown immunoreactive with a specific immunostain for zygomycetes (high magnification).

Infection (blastomycosis) occurs by inhalation of the fungus from its natural soil habitat. Infection may involve virtually any organ including the lungs, skin, bones, and brain (Fig. 4.15).

- *Coccidioides.* This fungus presents with endospores contained within thick-walled spherules that vary in size (20–150 μm). Endospores measuring 3–5 μm may be seen scattered singly if the spherule ruptures. When free endospores occur within macrophages, they can imitate other intracellular yeasts. The causative agents of infection (coccidioidomycosis) are *C. immitis* and *C. posadasii*. These fungi are endemic in American deserts. Infection causes granulomatous and miliary disease affecting largely the lungs. In endemic regions, fungus balls may develop within lung cavities.

TABLE 4.6. Morphology of commonly encountered yeasts and yeast-like cells.

Fungi	Yeast appearance	Yeast shape	Yeast size (μm)	Associated elements	Budding	Location
Candida		Oval	3–5	Pseudohyphae Rare hyphae	Narrow based	Mainly extracellular
Histoplasma		Oval to round	2–4	No hyphae	Narrow based	Mainly intracellular
Cryptococcus		Oval to round	5–15	Very rare pseudohyphae	Narrow based	Extracellular and intracellular
Blastomyces		Spherical	8–15	No hyphae	Broad based	Mainly intracellular

Coccidioides		Oval	3–5	Within spherules	None	Mainly extracellular
Sporothrix		Round to elongated	3–5	Rare hyphae	Narrow based	Mainly extracellular
Pneumocystis		Round to crescent	5–8	No hyphae	None	Mainly extracellular
Penicilliosis		Round to elongated	2–3	Rare hyphae	None	Mainly intracellular

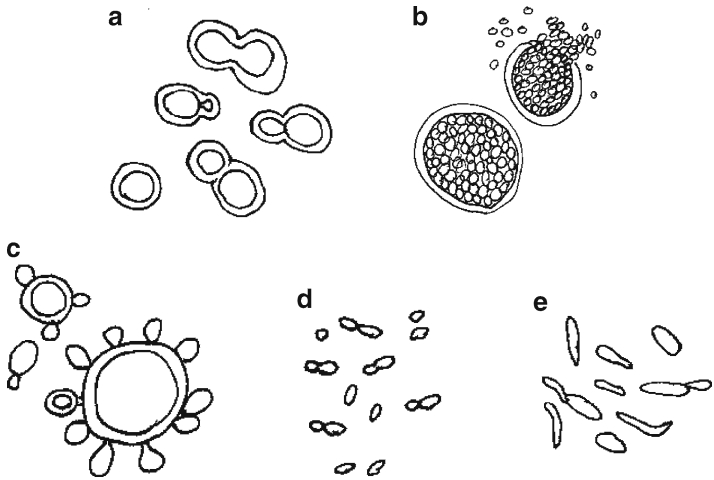


FIG. 4.15. Dimorphic fungi including (**a**) *Blastomyces dermatitidis*, (**b**) *Coccidioides immitis*, (**c**) *Paracoccidiodes brasiliensis*, (**d**) *Histoplasma capsulatum*, and (**e**) *Sporothrix schenckii.*

- *Paracoccidioides.* These yeasts measure 5–30 μm in size and are round to oval. Budding is characterized by a central yeast with multiple surrounding daughter buds, that morphologically resembles a "ship's wheel." Infection (paracoccidioidomycosis) is caused by *Paracoccidioides brasiliensis*, typically found in Brazil and elsewhere in South America. Primary infection (Valley Fever) is usually mild and self-limiting, but may progress into a systemic mycosis producing oral lesions, generalized lymphadenopathy, and miliary pulmonary lesions. Infection can also spread to bones, meninges, and the spleen.
- *Histoplasma.* This budding yeast is round to oval, on average 1–5 μm in size and observed mainly within macrophages (Fig. 4.16), but sometimes also within neutrophils. Narrow based round to oval budding may be noted, but because of their small size buds are often not seen. Intracellular yeasts are usually surrounded by a clear zone (halo). However, with cell disruption organisms may be spilled extracellularly. This fungus is usually found in bird and bat (guano) fecal material. There are a few

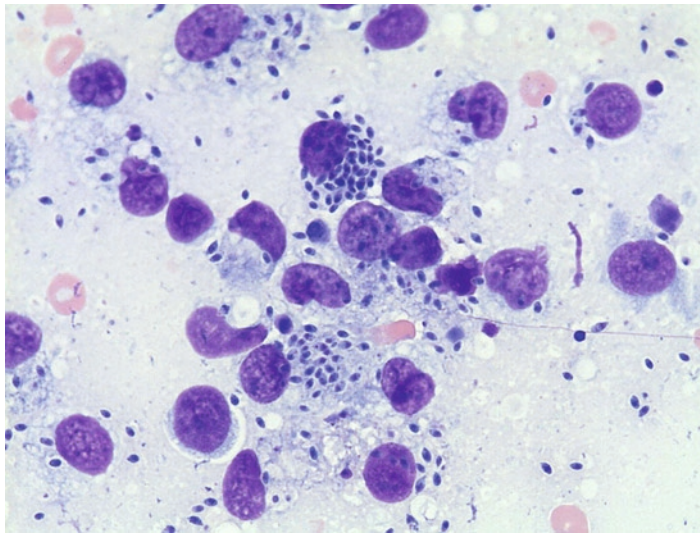


FIG. 4.16. Histoplasmosis. Numerous small intracellular and extracellular yeasts of *H. capsulatum* are shown (GMS stain, high magnification).

species including *H. capsulatum*. *H. capsulatum var. capsulatum* are smaller (1–5 μm) than *H. capsulatum var. duboisii* which have larger sized budding yeast cells (5–12 μm). Although this fungus occurs worldwide, it is prevalent in certain regions (Ohio and Mississippi river valleys) of North America and in caves of southern and East Africa. Infection causes histoplasmosis which primarily affects the lungs and mediastinum, but may become disseminated presenting with hepatosplenomegaly, lymphadenopathy, ocular and skin disease, as well as enlarged adrenal glands.

- *Sporothrix.* Yeasts measure 3–5 μm in diameter, are round to cigar-shaped, and can show single or multiple buds. Rarely aseptate hyphae may be observed. The only active species is *Sporothrix schenckii*, which is the causative agent of sporotrichosis (rose-handler's disease). Initial cutaneous infections may spread via lymphatics and disseminate to joints, bones, and the central nervous system. Inhaled fungi can cause pulmonary sporotrichosis with lung nodules, cavities, fibrosis, and hilar lymphadenopathy.

- *Penicillium.* These fungi produce penicillin. *Penicillium marneffei* is the only known thermally dimorphic species. In cytology specimens, Penicillium present in the mold phase with septate and branched hyphae represent a contaminant. However, in immunosuppressed patients *P. marneffei* is an opportunistic infection that causes penicilliosis. There is a particularly high incidence of penicilliosis in AIDS patients from tropical Southeast Asia. Infection after inhalation spreads from the lungs to involve the hematopoietic system and skin. Yeast-like cells are present within macrophages and extracellularly. They are not true yeast cells, but rather arthroconidia. Intracellular "yeasts" measure 2–3 μm in diameter and are round to oval. Because they divide by binary fission, budding is not observed. The extracellular organisms tend to be more elongated, sometimes up to 13 μm, and can have "septae" (crosswalls from binary fission).

Pneumocystis

- *Pneumocystis jirovecii* (previously called *Pneumocystis carinii*) is a yeast-like fungus of the genus *Pneumocystis*, which is the causative organism of *Pneumocystis pneumonia* (or pneumocystosis, formerly referred to as PCP).
- The cysts often collapse forming crescent-shaped bodies.
- All stages of the life cycle are found within the lung alveoli. Once inhaled, unicellular trophozoites (1–4 μm, Giemsa positive) undergo binary fission to form a precyst (difficult to distinguish by light microscopy) and ultimately develop thick walled cysts (5–8 μm, GMS positive). Spores (eight) form within these cysts, which are eventually released on rupture of the cyst wall.
- This organism is often seen in the lungs of healthy individuals, but is an opportunistic pathogen in immunosuppressed people, especially those with AIDS.
- Extrapulmonary disease may be seen with advanced HIV infection presenting with involvement of the lymph nodes, spleen, liver, bone marrow, gastrointestinal tract, eyes, thyroid, adrenal glands, kidneys, and within macrophages in pleural effusions (Fig. 4.17).

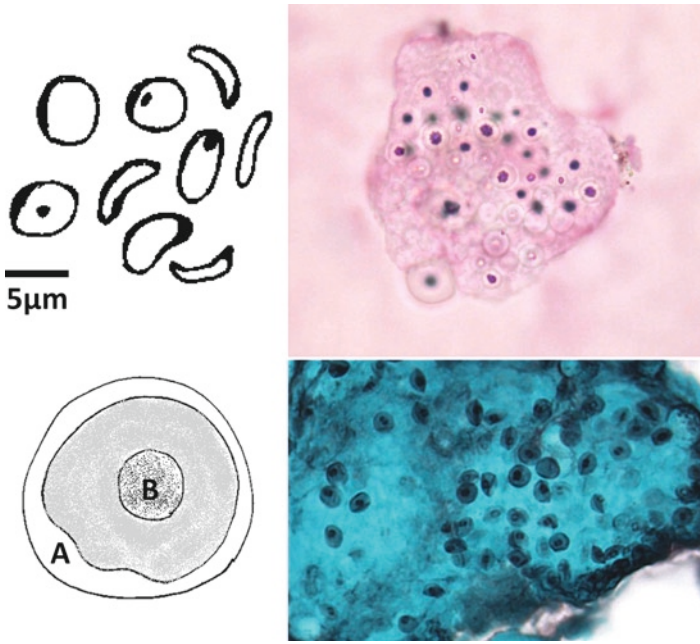


FIG. 4.17. *Pneumocystis*. (*Top left*) Diagram illustrating *P. jirovecii* thick walled cysts with a diameter (5 µm) approximately equal to that of a red-blood cell. When collapsed, some cysts assume a cup or crescent shape (crushed ping-pong ball appearance). (*Bottom left*) Schematic showing the ultrastructure of a cyst: The cyst wall is composed of an outer and inner layer that is focally thickened (**a**). The center of the cyst contains a trophozoite (**b**) with an ill-defined nucleus. (*Top right*) Bronchial washing from a patient showing a foamy alveolar cast containing *Pneumocystis* cysts (Pap stain, high magnification). (*Bottom right*) Localized areas of cyst wall thickening are best seen with a GMS stain also highlighting their central trophozoites (high magnification).

Dematiaceous Fungi

- The dematiaceous (naturally pigmented) group of fungi produce melanin in their cell walls. As a result, fungal colonies are brown when cultured and in tissue samples fungal forms are characteristically pigmented. A Fontana-Masson stain can be used to confirm the presence of fungal melanin pigment.

- They cause several human infections including chromoblastomycosis (also called chromomycosis) and phaeohyphomycosis (or phaeomycotic cyst).

Dermatophytes

- Dermatophytes cause infections of the skin and hair (ringworm or tinea) as well as the nails (onychomycosis). The three genera that cause these diseases include *Microsporum*, *Epidermophyton*, and *Trichophyton*.
- A rapid scraping of the nail, skin, or scalp can be used to identify characteristic hyphae and sometimes spores associated with squamous cells or within broken hairshafts.

Hyalohyphomycoses

- Hyalohyphomycosis is the term used to group together invasive mycotic infections caused by hyaline septate hyphae. This includes species of *Aspergillus*, *Penicillium*, *Paecilomyces*, *Acremonium*, *Beauveria*, *Fusarium*, and *Scopulariopsis*. They may represent contamination or cause invasive disease in the immunosuppressed host.
- *Fusarium* hyphae are similar to those of *Aspergillus*, with septate hyphae that branch at acute and right angles. Sporulation may also occur in tissue with infection (fusariosis). Their macroconidia are crescent-shaped, orangeophilic, and septate structures that measure 80–120 × 3–6 μm in size.

Parasites

Protozoa

- Protozoa are unicellular motile organisms. They are traditionally divided according to their means of locomotion such as amebae, flagellates, and ciliates.
- Their life cycle often alternates between trophozoites (feeding–dividing stage) and cysts (dormant stage able to survive outside the host). Their characteristics (particularly the nuclei and cytoplasmic inclusions) help in species identification. Ingested cysts cause infection by excysting (releasing trophozoites) in the alimentary tract.

- *Amebae* (Sarcodina) pathogenic to humans include intestinal and free-living amebae.
  - *Intestinal amebae. Entamoeba histolytica* causes amebiasis that may manifest with dysentery, flask-shaped colon ulcers, a colonic ameboma, and possible extraintestinal abscesses that contain anchovy paste-like material within the liver, and infrequently spleen or brain. Several of the amebae such as *Entamoeba dispar* are harmless and some may be relatively common, such as *Entamoeba gingivalis* that is usually found in the mouth. Unlike other amebae, the cytoplasm of pathogenic *E. histolytica* contains ingested red blood cells.
  - *Free-living amebae.* These include *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. *Acanthamoeba* cause granulomatous amebic encephalitis (GAE), contact lens-associated *Acanthamoeba* keratitis, and skin lesions. *Balamuthia* also causes GAE. *N. fowleri* ("brain-eating" ameba) is associated with rapidly fatal primary amebic meningoencephalitis (PAM), most often seen in children swimming in fresh water ponds and rivers during which amebae enter the nasal passages and migrate to the brain via the olfactory nerve. These trophozoites can be seen in CSF specimens, but culture on nonnutrient agar plates seeded with *Escherichia coli* and/or a flagellation test is required for confirmation.
- *Flagellates* (Mastigophora) are organisms that have one or more flagella.
  - *Giardia*. There are approximately 40 species described, but the species that infects humans is *Giardia lamblia* (also called *G. intestinalis* or *G. duodenalis*). This parasite is the most common cause of protozoal gastroenteritis (giardiasis). Trophozoites (9–21 μm long and 5–15 μm wide) are kite (or pear)-shaped and have two nuclei, four pairs of flagella, and two central axonemes running down their middle. Their cysts (8–14 × 7–10 μm) seen in stool specimens are oval, thick walled, and contain four nuclei and multiple curved median bodies.
  - *Trichomonas*. There are several trichomonad species such as the intestinal *Pentatrichomonas hominis* which is a nonpathogenic organism. *Trichomonas vaginalis* is the anaerobic, flagellated protozoan that causes trichomoniasis (Fig. 4.18). Details of this sexually transmitted infection are covered in Chap. 5. Apart from urogenital infections, *T. vaginalis* has

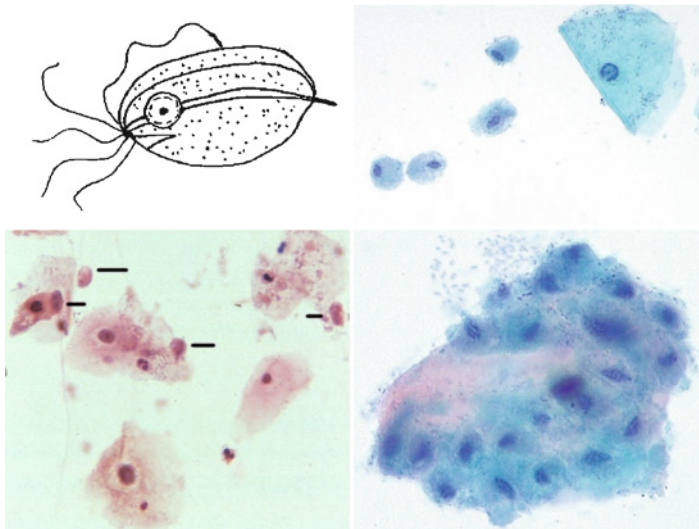


FIG. 4.18. *Trichomonas vaginalis*. (*Top left*) Illustration showing an oval organism (5–30 μm wide) that possess four anterior flagella, an undulating membrane, single large nucleus, and a central axostyle that projects from the posterior end. (*Bottom left*) *Trichomonas* organisms are shown in a sputum smear (indicated by *black bars*) with an oval shape and visible nuclei. Note that some of the squamous epithelial cells have typical perinuclear "trich halos" (Pap stain, high magnification) (image courtesy of Rafael Martinez Girón, Instituto de Piedras Blancas-Asturias, Spain). (*Right*) Several trichomonads are shown (*top right*) free in the background and (*bottom right*) attached to a squamous epithelial cell in a ThinPrep cervicovaginal Pap test (Pap stain, high magnification).

also been reported to cause pneumonia, bronchitis, and oral lesions. Pulmonary trichomoniasis is usually caused by aspirated *Trichomonas tenax* (mouth commensal) and less often *T. vaginalis* infection. An association between flagellated protozoa and asthma has been reported.

- *Leishmania*. These parasites are acquired from the sandfly. Depending on the species, they may cause cutaneous (e.g., oriental sore), mucocutaneous (espundia or uta), or visceral (kala-azar) leishmaniasis (Table 4.7). There are two morphological forms: promastigote (with a flagellum) found in

TABLE 4.7. Comparison of skin, mucocutaneous, and visceral leishmaniasis.

Type of infection	Pathogenic species		Geographic location
	Old world	New world	
Cutaneous	*L. major* *L. tropica* *L. aethiopica*	*L. mexicana*	South America, Middle East; North America (Southwestern USA)
Mucocutaneous	None	*L. braziliensis*	South America, China
Visceral	*L. donovani complex*	None	All continents except Australia

the insect host and an amastigote (without flagella) present in the human host (Fig. 4.19). Diagnostic samples may be procured from skin lesions (cutaneous or mucocutaneous) or bone marrow aspirates (visceral). The morphologic hallmark is the presence of multiple small (2–5 μm) intracellular amastigotes within macrophages (Leishman-Donovan bodies). Amastigotes are spherical to ovoid in shape and have both a nucleus and ovoid or rod-shaped kinetoplast. The differential diagnosis for multiple small organisms within histiocytes includes *H. capsulatum* (with budding and only intracellular) and toxoplasmosis (more curved and mostly extracellular).

  - *Trypanosoma*. The major human diseases caused by trypanosomatids are African trypanosomiasis (sleeping sickness) caused by *Trypanosoma brucei* and American trypanosomiasis (Chagas disease) caused by *T. cruzi*. Trypomastigotes (30 μm in length) are usually found in peripheral blood. They have an undulating membrane, central nucleus, and kinetoplast at the anterior end.

- *Ciliates* (Ciliophora) include the parasitic species *Balantidium coli*, the only member of this phylum known to be pathogenic to humans. The trophozoite is relatively large (50–70 μm), has a ciliated surface, and contains a kidney bean-shaped macronucleolus. The cyst form may occasionally also have cilia. Cilicytophthoria may be sometimes mistaken for these ciliated organisms.

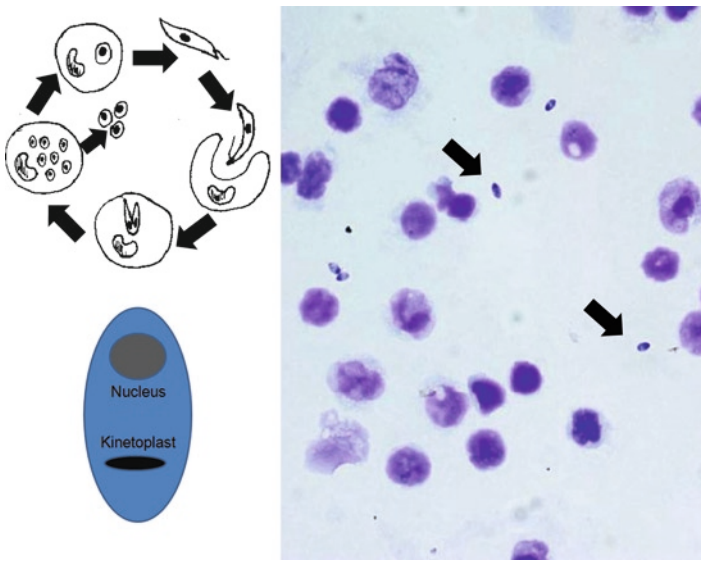


FIG. 4.19. *Leishmania*. (*Top left*) Life cycle showing the transition from a flagellated promastigote that occurs in the sandfly to small amastigotes without flagella in the human host: Promastigotes phagocytosed by macrophages multiply within these cells and disseminate when released. (*Bottom left*) Illustration of an ovoid amastigote shows a large nucleus and prominent rod-shaped kinetoplast. (*Right*) This FNA sample obtained from a Saudi Arabian child presenting with hepatosplenomegaly and lymphadenopathy from kala-azar shows few scattered amastigotes (*arrows*) among chronic inflammatory cells (Giemsa stain, high magnification).

Apicomplexans

- The Apicomplexa are a diverse group of protists that includes organisms such as coccidia (Sporozoa), *Plasmodium* spp. (cause malaria), and *Babesia* (cause babesiosis). Malaria and babesiosis are not discussed further because the diagnosis and speciation of these organisms primarily requires examination of peripheral blood smears and monoclonal antibody tests.
- Coccidian diseases include cryptosporidiosis (*Cryptosporidium* spp.), isosporiasis (*I. belli*), cyclosporiasis (*Cyclospora cayetanensis*), sarcocystis, and toxoplasmosis (*Toxoplasma gondii*).

The microsporidia, at one time a separate group (not coccidian), are now classified with the fungi.

- *Cryptosporidium* spp. *C. parvum* is the causative agent of cryptosporidiosis in humans and animals, a major cause of protracted diarrhea in patients with AIDS. Other species that cause human disease include *C. hominis*. These small (8–15 μm) oval parasites are identified within the brush border of the intestinal epithelium, and are discussed in the chapter on gastrointestinal infections. Ultrastructural studies have shown them to be intracellular but with an extracytoplasmic localization in enterocytes. Modified acid-fast thick-walled oocysts (4–6 μm) may be detected in stool samples. *Cryptosporidium* can disseminate beyond the intestine, especially in patients with AIDS, to involve the biliary tract, stomach, lungs, middle ear, and pancreas.
- *Microsporidia* include *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* (previously *Septata intestinalis*). Although these organisms are now considered fungi, parasitologists still maintain them in most books. In cytology specimens obtained from the small intestine, microsporidia appear as numerous small intracellular organisms within the apical portion of enterocytes. They stain well with Gram and silver stains (e.g., Warthin-Starry). Their spores (1–1.5 μm) may be found with a modified trichrome stain in stool samples as well as urine.
- *I. belli* and *Sarcocystis* infections very rarely have trophozoites that are detected. Their oocysts, however, may be seen when excreted in feces.
- *T. gondii* belongs to the genus *Toxoplasma*. Although infection can be acquired from the accidental ingestion of infective oocysts from cat feces (definitive host), most infections are acquired from eating infected rare or raw meats. Disease ranges from mild flu-like illness to fatal fetal infections. Latent infection may reactivate in immunosuppressed patients. Organisms may be found in samples from the brain, heart, eye, hematopoietic system, and lungs. Specimens may contain free (extracellular) tachyzoites which are small (3–5 μm), curved (banana-shaped) forms (Fig. 4.20). When parasites accumulate within macrophages (so-called “bag of parasites”) they form a pseudocyst (parasitophorous vacuole) containing bradyzoites.

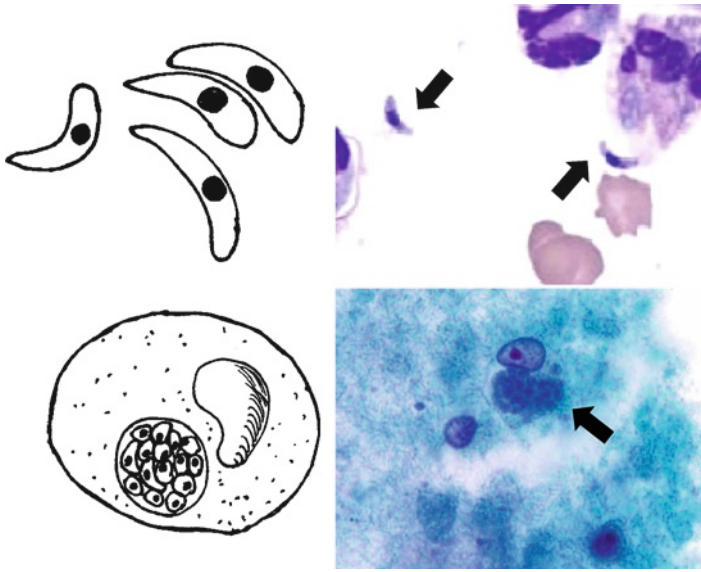


FIG. 4.20. *Toxoplasma gondii.* (*Top left*) Diagram showing crescent shaped tachyzoites (sometimes called endozoites) with a prominent nucleus: These trophozoites usually have a tapered anterior end and more blunt posterior end. (*Top right*) Free tachyzoites (*arrows*) can be seen in this BAL specimen (Giemsa stain, high magnification). (*Bottom left*) Diagram showing a macrophage with a pseudocyst containing multiple bradyzoites: Note that some of these microorganisms are still crescent shaped. The cysts are usually round in brain tissue, more elongated in muscle, and often very small and hard to identify in lung tissue. (*Bottom right*) An infected macrophage contains a cluster of bradyzoites (Pap stain, high magnification).

Helminths

- Helminths (parasitic worms) are categorized into three groups: cestodes (tapeworms), nematodes (roundworms), and trematodes (flukes) (Table 4.8). Flukes and tapeworms belong to the phylum platyhelminthes (flatworms).
- Clinical infection may be caused by adult worms, larvae, and/or eggs (Fig. 4.21). Infections are usually diagnosed by the characteristics of these different developmental stages.

TABLE 4.8. Common parasitic worms (helminthiases).

Helminth	Worm	Egg
Cestodes (tapeworms)		
Taenia saginata (beef tapeworm)	Scolex with four suckers and proglottids	Radially striated wall (30–40 μm)
Taenia solium (pork tapeworm)	Scolex with four suckers, hooklets and proglottids	Radially striated wall (30–40 μm)
Diphyllobothrium latum (fish tapeworm)	Scolex with wide proglottids	Oval with operculum and knob at either end (up to 60 μm)
Hymenolepis nana (dwarf tapeworm)	Very small (2–4 cm)	Wide inner and outer shells (30–47 μm), contain polar filaments
Echinococcus spp.	Protoscolices in hydatid cyst	Identical to *Taenia* (30–45 μm)
Nematodes (round worms)		
Trichuris trichiura (whipworm)	Whip-like anterior end	Barrel shaped with polar plugs at both ends (20–50 μm)
Ascaris lumbricoides	Large (up to 35 cm)	Rough mammillated shell (up to 75 μm)
Necator americanus (hookworm)	Mouthpart with cutting plates (adult worm)	Thin wall with internal morula (35–75 μm)
Ancyclostoma duodenale (hookworm)	Mouthpart with teeth (adult worm)	Thin wall with internal morula (35–75 μm)
Strongyloides stercoralis	Shorter buccal groove (mouth) than hookworm (rhabditiform larvae)	Identical to hookworm (35–75 μm), rarely seen
Enterobius vermicularis (pinworm)	Pointed pin-like tail	One side flattened (20–60 μm)
Trematodes (flukes)		
Fasciola hepatica (liver fluke)	Flat with cephalic cone	Very large (up to 150 μm), operculated (cannot distinguish from *Fasciolopsis buski*)
Fasciolopis buskii (intestinal fluke)	Flat with pointed head	Very large (up to 150 μm)
Clonorchis sinensis (liver fluke)	Flat with snout-like head	Small with shouldered operculum (12–20 μm)
Paragonimus westermani (lung fluke)	Flat ovoid worm	Oval with shouldered operculum (45–120 μm)

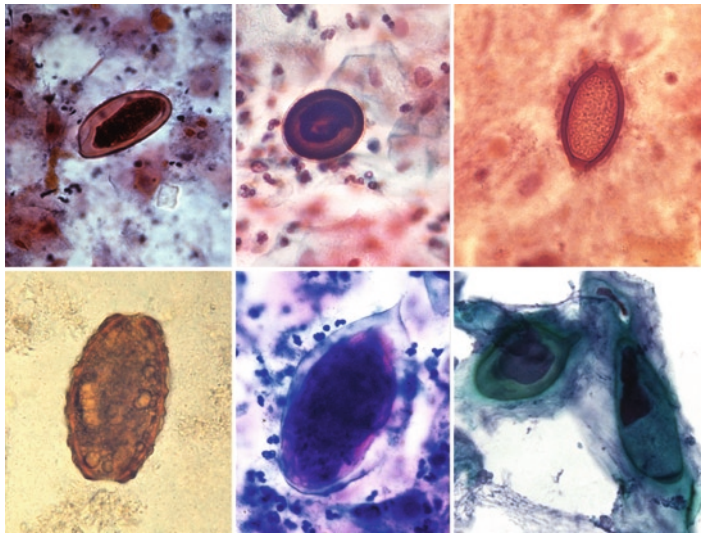


FIG. 4.21. Parasitic eggs are shown in cytologic preparations. (*Top left*) *Enterobius vermicularis.* (*Top middle*) *Taenia* (tapeworm). (*Top right*) *Trichuris trichiura*. (*Bottom left*) *Ascaris lumbricoides*. (*Bottom middle*) *Schistosoma haematobium*. (*Bottom right*) Pollen grain (belonging to the *Caryophyllaceae* family) that may be mistaken for *Toxocara* eggs (images courtesy of Dr. Pam Michelow, South Africa and Dr. Rafael Martinez Girón, Spain).

- The host response to these parasites includes eosinophilia, acute inflammation, and sometimes granulomas.
- *Cestodes*. These tapeworms usually parasitize humans after eating underprepared meat such as pork (*Taenia solium*), beef (*Taenia saginata*), or fish (*Diphyllobothrium* spp.), or food that is prepared in conditions of poor hygiene (e.g., *Echinococcus* spp.).
  - *Taenia*. Infection with the adult tapeworms occurs following ingestion of cysticerci in rare or poorly cooked beef or pork. *T. saginata* (beef) infection results in small intestinal infestation of adult worms (taeniasis). *T. solium* (pork) infection can also cause cysticercosis, as a result of the accidental ingestion of food or drink contaminated with tapeworm eggs from the feces. Cysticercosis is characterized by cysts

containing larvae located in several sites such as the brain (neurocysticercosis causes seizures), eye, as well as muscles and subcutaneous tissue (causes painful nodules). These cysts may cause eosinophilia, an inflammatory reaction and eventually they may calcify.

- *Echinococcus*. Infection from the accidental ingestion of food or drink contaminated with tapeworm eggs results in hydatid disease, also known as echinococcosis. The larval cysts that develop can be found in virtually any site, grow slowly, and persist for many years until they cause symptoms or are discovered incidentally. Disruption of a cyst containing highly antigenic fluid may result in anaphylactic shock, and for this reason it has been recommended that they should not be biopsied. However, on the basis of published data adverse reactions are rare. Use of a fine gauge needle by a skilled operator is important to prevent fluid leakage during aspiration. There are three different forms of echinococcosis: cystic (unilocular) echinococcosis (caused by *Echinococcus granulosus*), alveolar (multilocular) echinococcosis (caused by *Echinococcus multilocularis*), and polycystic echinococcosis (caused by *Echinococcus vogeli* and rarely *Echinococcus oligarthus*). A hydatid cyst contains a thick outer (acellular) wall and thin inner germinal epithelium. Alveolar and polycystic echinococcosis cysts usually have multiple compartments. Hydatid cysts may contain several liters of fluid, daughter cysts, and hydatid sand (Fig. 4.22). Aspirated hydatid sand contains protoscolices (future scolices) and free hooklets. Viable protoscolices typically show a row of parallel hooklets whereas dead ones contain haphazardly attached hooklets.

- *Nematodes.* There are several roundworms (Table 4.7), but those likely to be seen in cytology specimens are *Enterobius vermicularis*, *Strongyloides stercoralis*, and the microfilariae.
  - *E. vermicularis* (pinworm, also known as the threadworm in the United Kingdom) causes intestinal infestation (enterobiasis). The adult female worm migrates out onto the perianal area at night, and once exposed to oxygen she lays her eggs. This causes perianal pruritis and possibly vaginitis. Occasionally subcutaneous perineal nodules may develop. In such cases, Pap tests may contain an adult worm and/or eggs. The worm has a

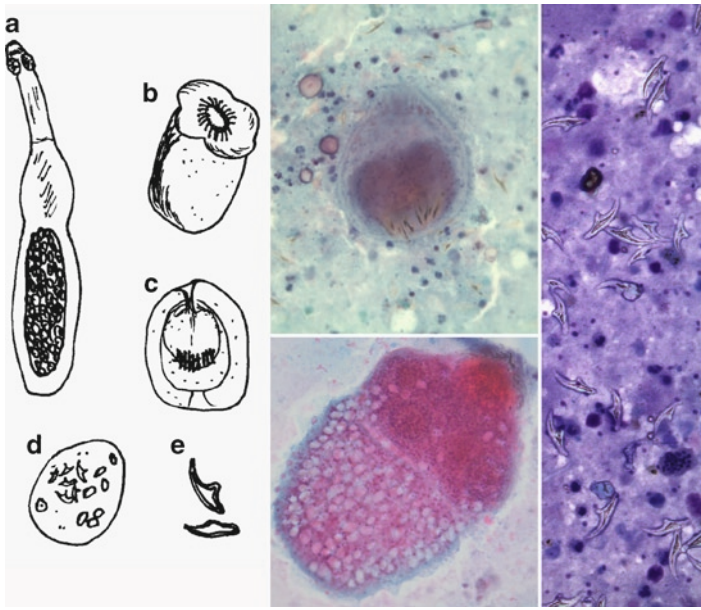


FIG. 4.22. *Echinococcus granulosus*. (*Left*) (**a**) Diagram showing an adult tapeworm that consists of a scolex (head) and three proglottids. The scolex has suckers and a crown of hooklets (adult worm not found in humans). Components of hydatid sand are illustrated including (**b**) evaginated protoscolex (usually occurs when placed into saline), (**c**) invaginated protoscolex with hooklets, (**d**) degenerated scolex with calcareous corpuscles, and (**e**) individual rostellar hooklets. (*Top middle*) Invaginated protoscolex (Pap stain, high magnification). (*Bottom middle*) Evaginated protoscolex (Pap stain, high magnification). (*Far right*) Free sickle shaped hooklets (MGG stain, high magnification) (cytology images courtesy of Pam Michelow and Dr. Pawel Schubert, South Africa).

characteristic pointed tail (Fig. 4.23). Their eggs are colorless, oval, thin walled, and flattened on one side,

- *S. stercoralis*. This worm causes strongyloidiasis. After penetrating the skin, infective larvae pass through the lung (Loeffler syndrome). They are then coughed or swallowed and infest the duodenum. Here new autoinfective larvae may develop (autoinfection) leading to chronic infection. In immunocompromised patients, larvae may penetrate the intestinal wall

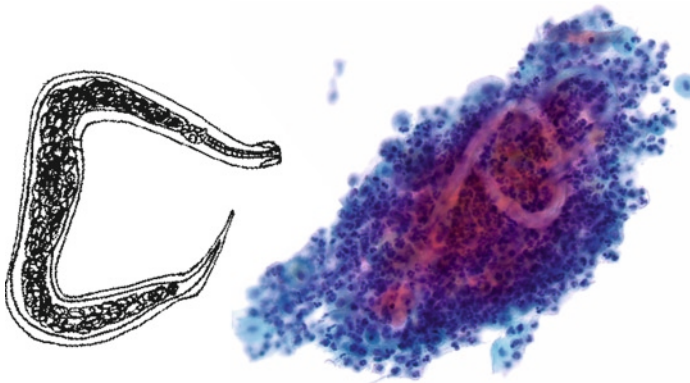


FIG. 4.23. *E. vermicularis* (pinworm). (*Left*) Diagram showing an adult female worm (8–33 mm long × 0.3–0.5 mm wide) that has a cephalic inflation and characteristic long, pointed tail. (*Right*) Pinworm shown among neutrophils in a Pap test. If the morphology of the worm is not definitive, then a diagnosis reporting a worm-like structure suggestive of pinworm is appropriate (Pap stain, intermediate magnification).

and disseminate via the bloodstream (called hyperinfection). Disseminated strongyloidiasis has a very high mortality rate. Cytology specimens including gastrointestinal and pulmonary (e.g., sputum) samples may contain larvae, either rhabditiform (noninfective) or filariform (infective) types.

- *Filariae.* These roundworms cause filariasis. Microfilariae that may be periodically found in the peripheral blood include *Wuchereria bancrofti*, *Brugia malayi*, and *Loa loa*. *W. bancrofti* and *B. malayi* infest lymphatics leading to lymphadentitis and chronic lymphedema (elephantiasis). *L. loa* resides in subcutaneous tissue and the conjunctiva. Certain microfilariae such as *Mansonella perstans*, *Onchocerca volvulus*, *Dracunculus medinensis*, and *Dirofilaria immitis* are not readily found in blood smears. *M. perstans* inhabits body cavities such as the peritoneum and pleura (serous cavity filariasis). *O. volvulus* infects subcutaneous tissue forming nodules and the conjunctivae causing blindness (onchocerciasis or river blindness). *D. immitis* (dog heartworm) causes granulomatous nodules in the lung. Microfilariae are the diagnostic forms of infection. The presence or absence of a sheath

and the pattern of nuclei in their tail are the main features used to distinguish the various species. Occult filariasis has been diagnosed by many bloody FNA procedures containing microfilariae, worms, or even eggs. Filarial morphology is best appreciated with a Giemsa stain. The background tissue response in cytology aspirates may include eosinophils, neutrophils, chronic inflammation, and even granulomas.

- *Trematodes.* The flukes are oval or worm-like helminthes that are parasites of molluscs and vertebrates. The liver flukes include *Fasciola hepatica* and *Clonorchis sinensis* that result in infestation of the bile ducts and subsequent biliary fibrosis. Infection with *C. sinensis* is a risk factor for cholangiocarcinoma. *Fasciolopis buskii* is an intestinal fluke that infests both the bile ducts and duodenum. *Paragonimus westermani*, the lung fluke, causes lung infestation with pulmonitis. Also included are the schistosomes (blood flukes).
  - *Schistosomes.* Infection by these trematodes causes schistosomiasis (bilharzia). There are three human schistosome pathogens: *Schistosoma mansoni* from Africa (Nile delta) which causes intestinal schistosomiasis, *S. haematobium* from Africa and the Middle East that infests the urinary bladder, and *S. japonicum* from China and Southeast Asia that primarily involves the liver. *S. haematobium* infection can lead to squamous cell carcinoma of the bladder. Microscopic identification of eggs in stool or urine specimens, as well as tissue biopsies, can provide a rapid diagnosis. The spines present on schistosome eggs help distinguish these different species. The egg of *S. haematobium* (oval) has a terminal spine, *S. mansoni* (oval) has a lateral spine, and *S. japonicum* (round) has a small knob-like lateral spine.

Algae

- Algae are ubiquitous and include a diverse group of simple organisms that range from unicellular to multicellular forms. The main algal groups include the cyanobacteria, green algae, and red algae (e.g., dinoflagellates).
- Most algae present in cytology samples are from contamination, discussed in greater detail in Chap. 15.

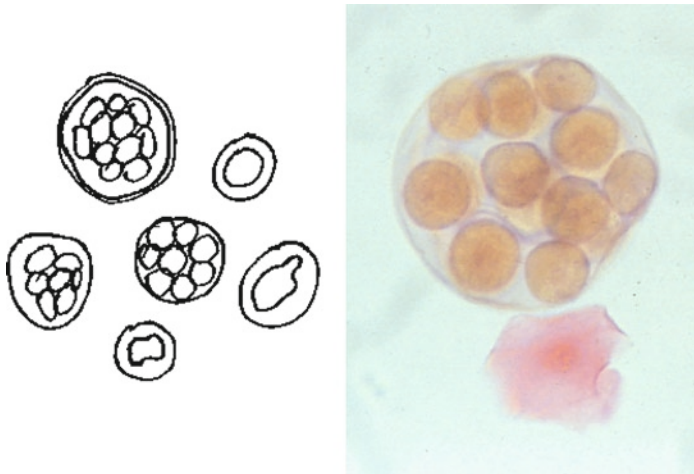


FIG. 4.24. *Prototheca*. (*Left*) Diagram of prototheca demonstrating variable morula formation: (*Right*) A sporangium of *P. wickerhamii* is shown in which endospores have a moruloid (daisy-like) pattern (Pap stain, high magnification) (image courtesy of Rafael Martinez Girón, Instituto de Piedras Blancas-Asturias, Spain).

- *Prototheca* is a genus of algae that lacks chlorophyll that causes the disease protothecosis. Most human cases are caused by *P. wickerhamii.* Infection may cause skin lesions or disseminated and systemic disease. Organisms may be seen within macrophages or extracellularly. They form spherical sporangia that range in size from 3 to 10 μm, have thick walls, and show no budding. These morular forms often contain endospores arranged symmetrically, typically in a daisy-like pattern (Fig. 4.24). They stain with most stains including H&E, PAS, and GMS stains.

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