3 Infection and Inflammation

Let us review the types of inflammatory responses you may see. It seems very basic, but learning to differentiate inflammatory changes from dysplastic ones is a fundamental goal in pathology training.

Acute

Acute changes are the result of recent tissue damage, either from trauma, ischemia, toxins, or infection. Features include the following:

- Vascular congestion
- Edema
- Fibrinous exudate
- Tissue damage and/or necrosis
- Neutrophils ("purulence", polymorphonuclear leukocytes, polys)

Acute inflammation can be followed by resolution (healing), fibrosis or scar, abscess formation (Figure 3.1), or a chronic inflammatory stage. Evidence of recent damage and reparative changes includes granulation tissue, hemosiderin, lipid-laden macrophages, and fibroblast proliferation.

Granulation tissue has a characteristic look of a watery or myxoid background with sparse fibroblasts floating in it and a proliferation of inflammatory cells (all types) and capillaries (Figure 3.2). The endothelial cells of the capillaries can become quite prominent.

Biopsy site changes, a term often used to indicate evidence of a recent procedure, include fibroblast proliferation (early scar), foreign body–type giant cells, suture material, foamy macrophages, fat necrosis, and inflammation. They have a more solid look to them than granulation tissue (Figure 3.3).

Scar tissue implies that a dense thick collagen has replaced the normal structures. In the skin, a dermal scar is evidenced by a homogeneous pink layer of collagen and absence of adnexal structures (Figure 3.4).

Chronic

Chronic changes are the result of repetitive or sustained tissue damage due to trauma, ischemia, toxins, infection, or autoimmune processes. Features include the following:

- Increased vascularity and/or fibrosis (attempts to heal)
- Tissue destruction
- Lymphocytes, macrophages, plasma cells, eosinophils

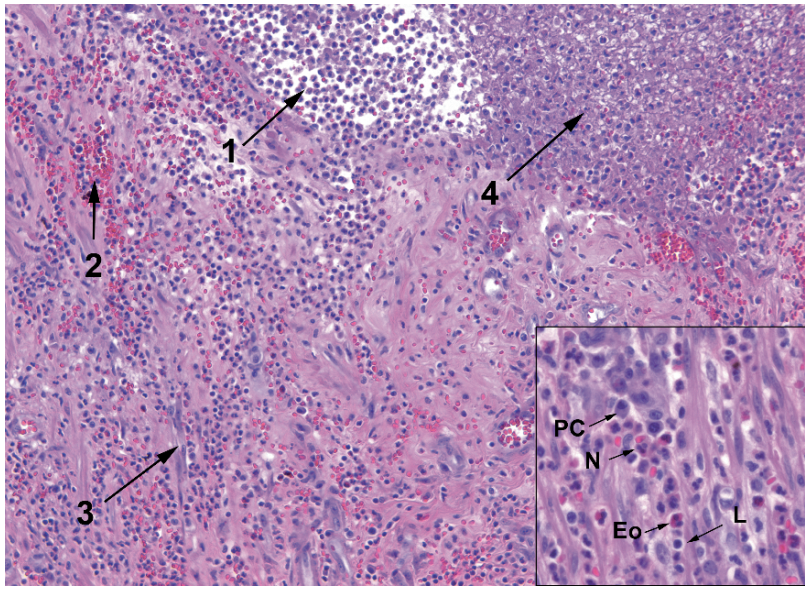

FIGURE 3.1. Acute inflammation and abscess formation. This example of the acute inflammatory response shows collections of neutrophils (abscess formation, 1), extravasated blood (2), prominent capillaries (3), and fibrin accumulation (4). **Inset**: the mixed inflammatory infiltrate includes plasma cells (PC), neutrophils (N), eosinophils (Eo), and lymphocytes (L).

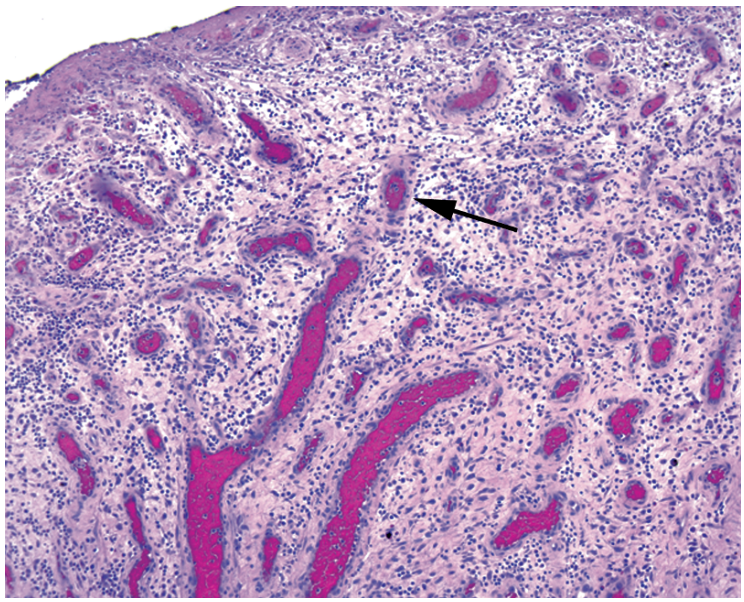

FIGURE 3.2. Granulation tissue is characterized by a loose myxoid background with fibroblasts and inflammatory cells and by prominent capillaries with plump endothelial cells and thick walls. The stroma appears condensed and thickened around the capillaries, giving them a pink halo (arrow).

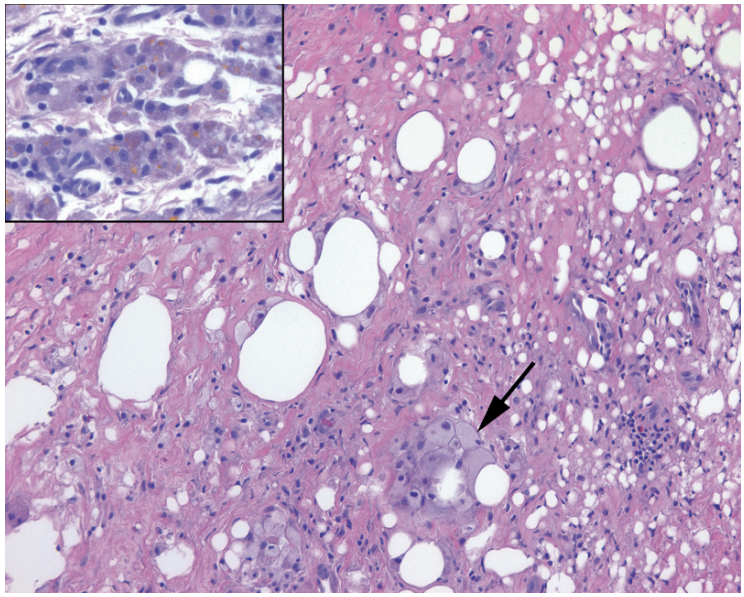

Figure 3.3. Biopsy site changes. In this subcutaneous specimen, collagen has replaced most of the fat cells, and foamy histiocytes can be seen ingesting some residual fat (arrow). **Inset**: Hemosiderin in macrophages (golden yellow to brown granules) can be seen in sites of prior trauma or bleeding.

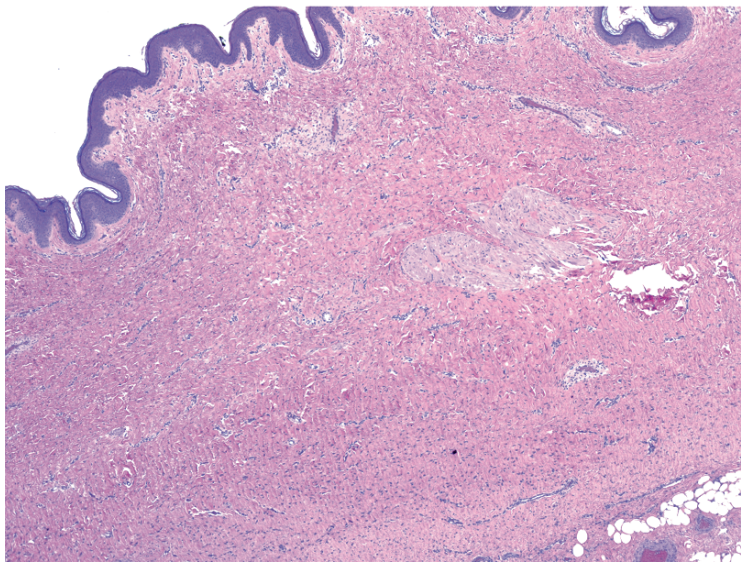

Figure 3.4. Dermal scar. Dense pink collagen has replaced the adnexal structures and displaced the subcutaneous fat in this biopsy site.

What is a *macrophage*? The precursor is a circulating monocyte, part of the myeloid lineage of blood cells (*myeloid* generally refers to cells in the granulocyte and monocyte groups, although it can also mean all cells that mature in the bone marrow, i.e., the opposite of lymphoid). The monocyte leaves the circulation and becomes a tissue macrophage. It can differentiate into organ-specific resident macrophages, such as microglia, Kupffer cells, and alveolar macrophages. It can also go to an area of inflammation and become activated, participating in the immune response. Activated macrophages are also called *histiocytes* and may be "epithelioid," as in a granuloma, or "foamy," as

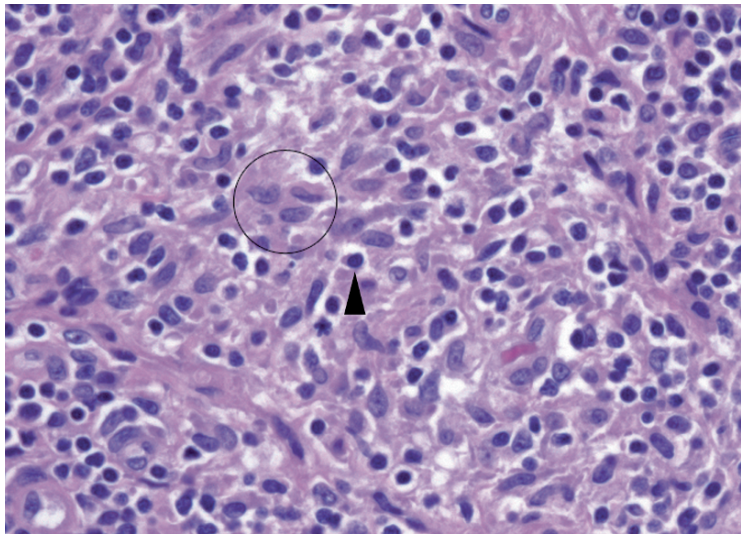

Figure 3.5. Histiocytes appear as pale folded nuclei within an area of inflammation; the cell borders are indistinct, but the nuclei are surrounded by light pink cytoplasm (circle). Compare the pale chromatin to that of the neighboring lymphocyte (arrowhead).

in lipid-laden or xanthomatous. Finally, macrophages can acquire multiple nuclei to become a Langerhans giant cell (ring of nuclei) or a foreign body–type giant cell (scattered nuclei).

Histologically, histiocytes have a bland and fade-into-the-background look to match their name (literally, "tissue cell"). They have pale-pink granular cytoplasm, sometimes with chunky phagocytosed bits of material, and indistinct cell borders (Figure 3.5). The nuclei are light with crisp outlines, oval in shape, and often grooved. In tissue, a collection of histiocytes appears as an ill-defined pink area that is easy to miss. The nuclei often stream in a circular pattern like fish swimming in a barrel. Foamy macrophages are stuffed with lipid debris or organisms, and can have an almost signet-ring appearance.

What are *eosinophils*? Eosinophils have a bilobed nucleus and big red granules. They are usually an indication of an immune/IgE response, such as to drug allergy or parasites.

We usually refer to the presence of lymphocytes as *chronic inflammation*. Lymphocytes plus neutrophils equals acute and chronic inflammation. In the gastrointestinal tract, instead of *acute* we use *active*, such as active chronic gastritis or active chronic inflammatory bowel disease. *Inactive* in the gastrointestinal tract means increased lymphocytes but no polys.

Granulomatous

A granulomatous appearance indicates a specific type of chronic inflammation with a small differential; it can be a result of mycobacteria (plus a few other bacteria), fungal infection, autoimmune disease, some toxins or irritants, and sarcoid. Granulomas are divided into caseating (usually infectious) and noncaseating.

The histologic appearance of a granuloma is a microscopic aggregate of histiocytes, with surrounding lymphocytes and plasma cells. The appearance ranges from tiny collections of histiocytes (as in Crohn's disease; Figure 3.6), to large well-circumscribed whorls of cells (sarcoid; Figure 3.7), to a layer of histiocytes surrounding a pool of caseous necrosis (tuberculosis, fungus; Figure 3.8). Giant cells are helpful but not essential. Old granulomas can become hyalinized and acellular (Figure 3.9).

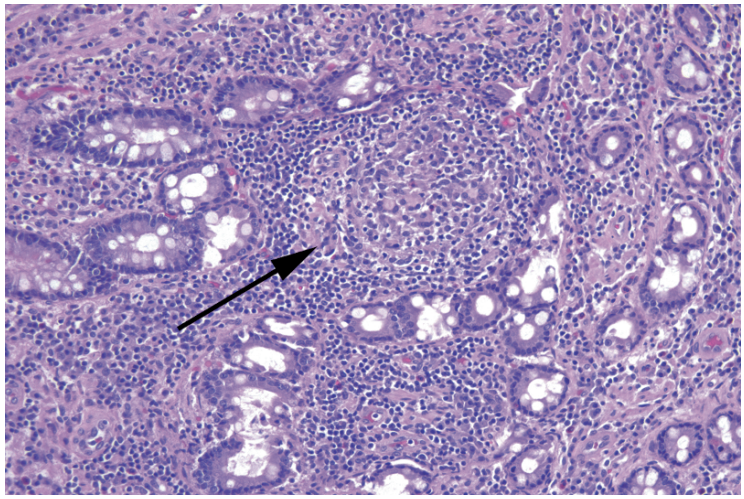

Figure 3.6. Granulomas in Crohn's disease. These granulomas of the colon are subtle (arrow), and the pale histiocytes may be seen only on high power. A surrounding collar of lymphocytes is common.

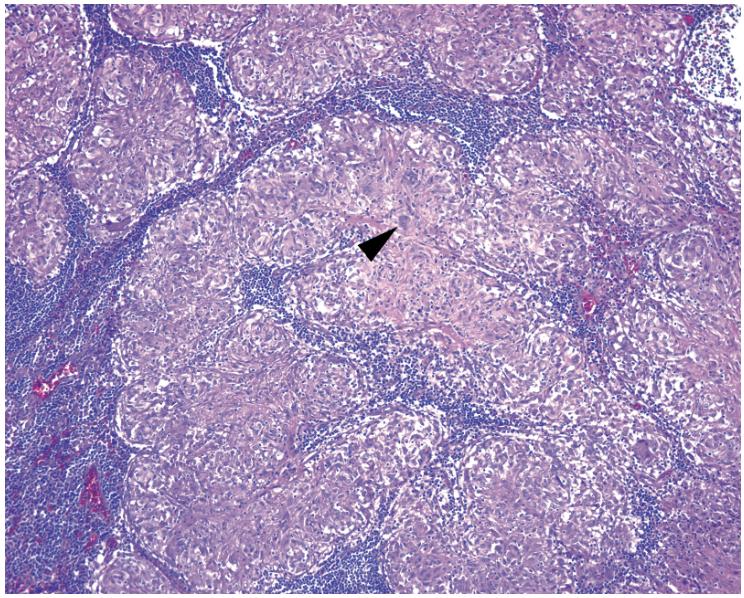

Figure 3.7. Granulomas in sarcoid. These granulomas are often more substantial and more easily recognized than those in Crohn's disease. They appear as well-defined masses of pink histiocytes. Occasional multinucleated giant cells (arrowhead) are present.

Specific Organisms and Their Stains

Fungi

Fungal organisms stain bright pink on periodic-acid Schiff (PAS) stain and black on Gomori's methenamine silver (GMS) stain. For most of these organisms, it is important to identify not just the presence and morphology of the organism but whether it is invading viable tissue or colonizing necrotic debris. Size can be helpful in identifying the various yeasts (Figure 3.10).

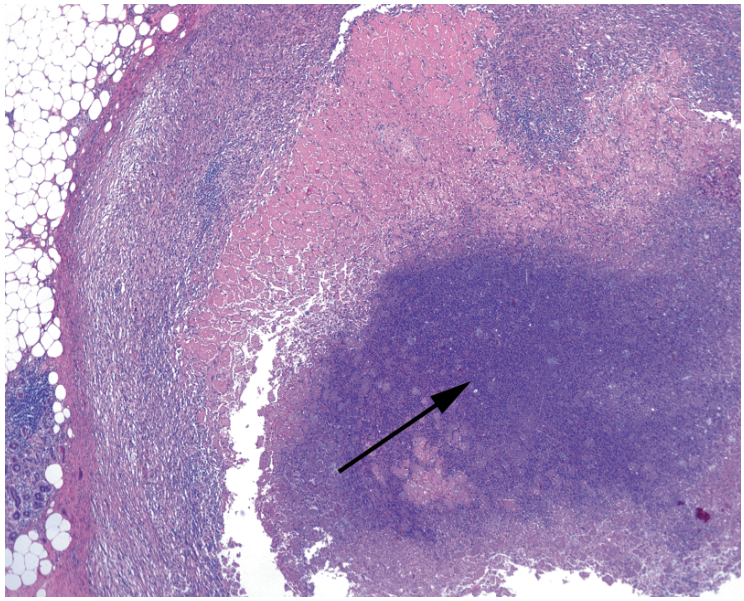

Figure 3.8. Caseating granulomas in tuberculosis. The histiocytes in these granulomas are visible only at the periphery, as the center is a mass of necrosis and cellular debris (arrow).

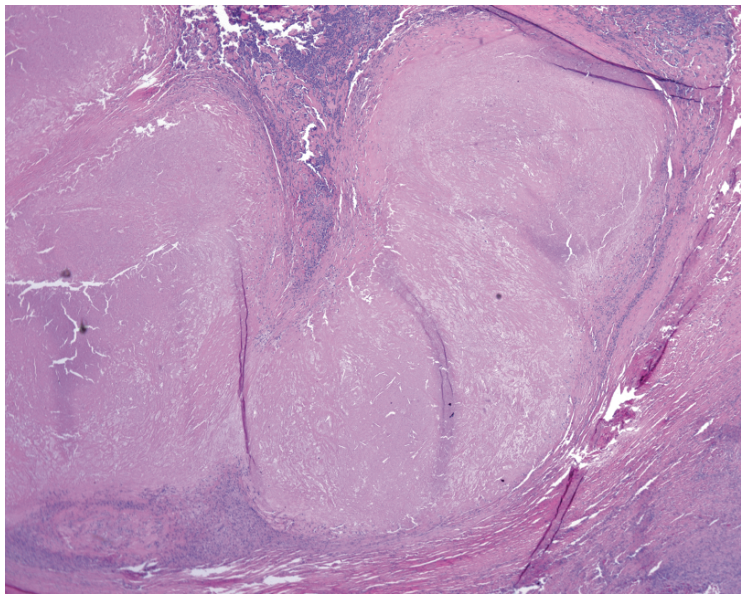

Figure 3.9. Hyalinized granuloma. The amorphous area of hyalinized collagen likely represents old, burned-out necrosis.

Candida are visible on H&E as round-to-oval yeast forms and pseudohyphae (segmented and nonbranching). They are often found in the debris at the epithelial surface (Figure 3.11).

Aspergillus are visible on H&E as long, thin hyphae with 45° branching and septations. They may appear as a solid fungal ball or as single hyphae in the tissue (Figure 3.12). Treated *Aspergillus* may have different morphology.

Mucor and zygomycetes are irregular and wide nonseptate hyphae and have the appearance of gnarled tree branch outlines with wide branch points (Figure 3.13). On H&E, they can be almost invisible, as they are essentially wide hollow spaces in the tissue. These are the bread

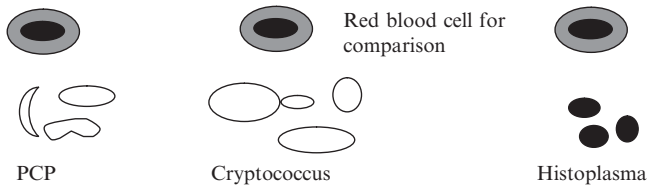

Figure 3.10. Relative size of yeasts.

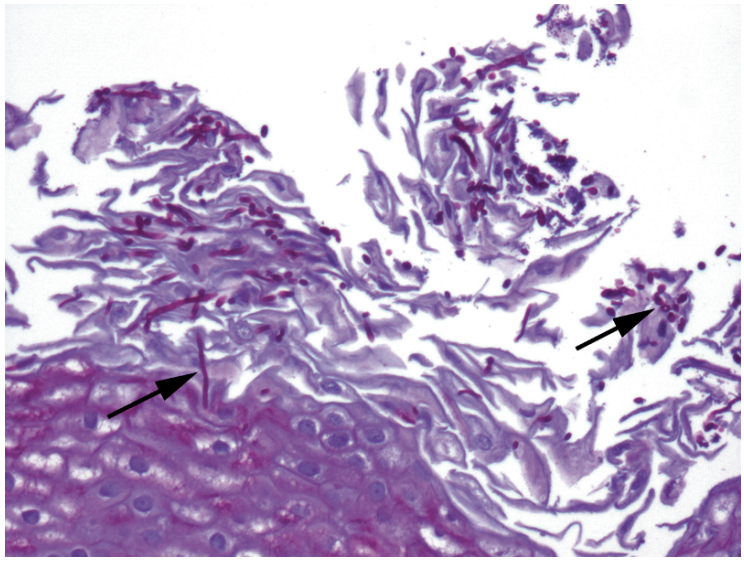

Figure 3.11. *Candida*. This example from the esophagus shows magenta pseudohyphae and yeasts (arrows, periodic-acid Schiff stain).

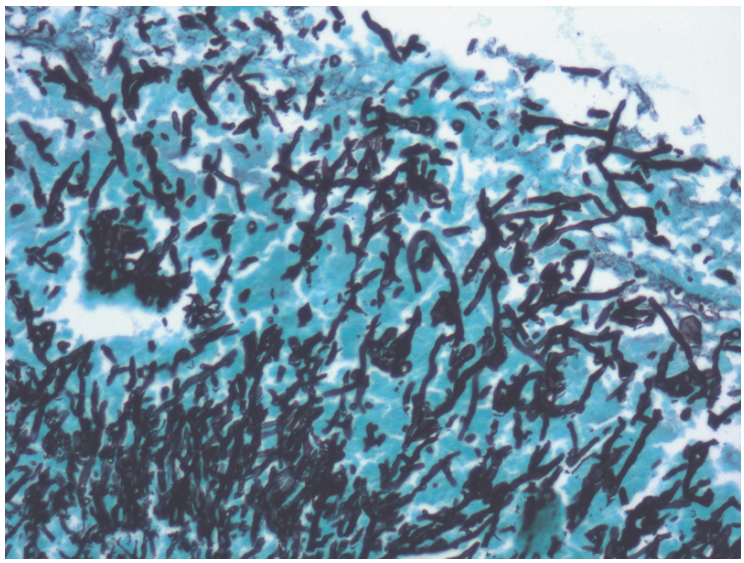

Figure 3.12. *Aspergillus*. A forest of branching hyphae are visible by Gomori's methenamine silver stain.

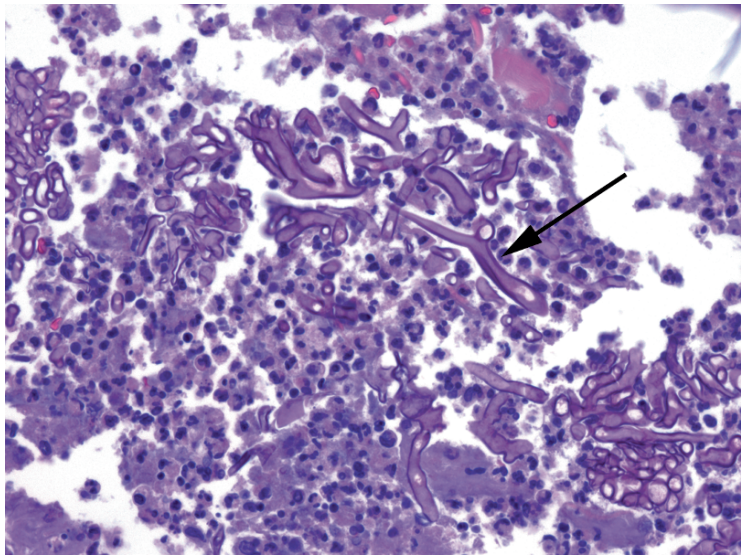

Figure 3.13. *Mucor*. A periodic-acid Schiff stain shows the thick, "hollow," irregular outlines of *Mucor* (arrow).

molds and are typically seen only in very neutropenic patients or in sinusitis in a patient with ketoacidosis.

Histoplasma are tiny intracellular yeast forms with narrow-based budding, often seen in macrophages. On H&E and Giemsa stain, these are delicate 2-µm forms in macrophages. In the context of a hyalinized granuloma, however, a silver stain shows distinct yeasts that are nearly the size of red cells (about 5 µm; Figure 3.14).

Cryptococcus are usually encapsulated yeast forms with narrow-based budding; some may be in macrophages but are often free in the tissue; on GMS the sizes are variable, and some may collapse into squashed balls (Figure 3.15). This variability in size is actually a key indicator of *Cryptococcus*. Stains for the capsule of *Cryptococcus* can differentiate it from other yeasts, including mucicarmine and Fontana-Masson. However, be aware that *Cryptococcus* can occasionally lose the capsule.

Pneumocystis may or may not be a fungus but are definitely black on GMS. They are flattened contact-lens–shaped organisms found in the alveoli (Figure 3.16). They are not visible on H&E but are usually accompanied by a foamy pink exudate.

Bacteria

Most bacteria are not found by, or identified with, stains. This is because there is little more we could say than "Gram-positive cocci in clusters," for example, which is pretty unhelpful without a culture. There are a few that are hard to culture and are best identified by stains.

Histologic evidence of *Mycobacterium* (causing tuberculosis and other diseases) is caseating granulomas. The organisms are not seen on H&E and may be very sparse in an immunocompetent patient. The conventional stain is the acid-fast (AFB) stain, which leaves the tissue unstained, with occasional pink blush in some cell types, but stains mycobacteria a bright wine red (Figure 3.17). These are tiny scattered bacilli; you need to be at 40×, at least, to spot them. Scanning the entire slide at 40× for red lint is painful but necessary to rule out infection. If clinical suspicion is high but an AFB is negative, an auramine-rhodamine is a more sensitive fluorescent stain for tuberculosis.

Mycobacterium avium-intracellulare causes infection in an immunocompromised patient. In these patients, the mycobacteria are eaten by macrophages and then multiply like crazy within the cells, giving the appearance of foamy macrophages. In the duodenum this can look just like Whipple's disease, but a PAS stain will differentiate the two (histiocytes stuffed with

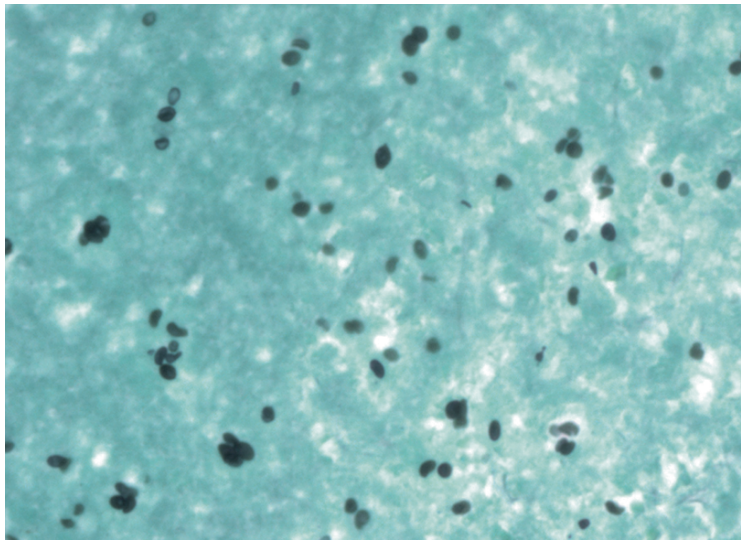

Figure 3.14. Histoplasmosis. Tiny yeasts are visible on Gomori's methenamine silver stain (40× objective).

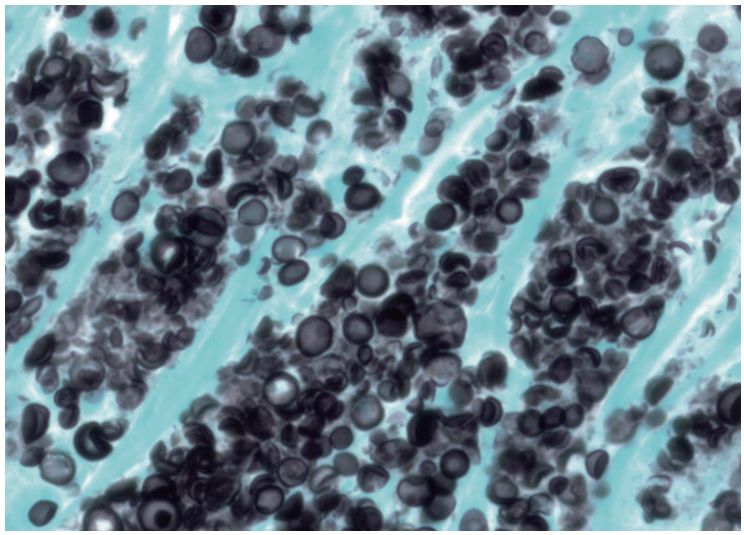

Figure 3.15. *Cryptococcus*. This photograph is taken at the same magnification as Figure 3.14. The organisms are significantly larger and show a range of sizes and shapes on Gomori's methenamine silver stain.

cranberries in Whipple's disease but with fine bacilli in *M. avium-intracellulare* infection). An AFB stain will also be positive.

Helicobacter pylori is the most common cause of gastric ulcers. Histologically you should see a chronic inflammatory infiltrate in the stomach, with a little activity here and there (polys). Infection is more common in the antrum. On Diff-Quik or Giemsa stain, look in the areas of activity. If present, *H. pylori* will be in the pit lumens or at the surface in clusters of tiny (barely visible at 20×) seagull-shaped bacilli (Figure 3.18). Sometimes you will hear them called *helicopters*. This is a phonetic joke, not a visual one. They do not look like helicopters.

Actinomyces, causing a puffball bacterial colony, is completely unremarkable in the tonsil but significant in endometrium, especially in the setting of an intrauterine device. The H&E appearance is of a granular grey-purple cloud, sometimes filamentous, with no identifiable cells or structures (Figure 3.19).

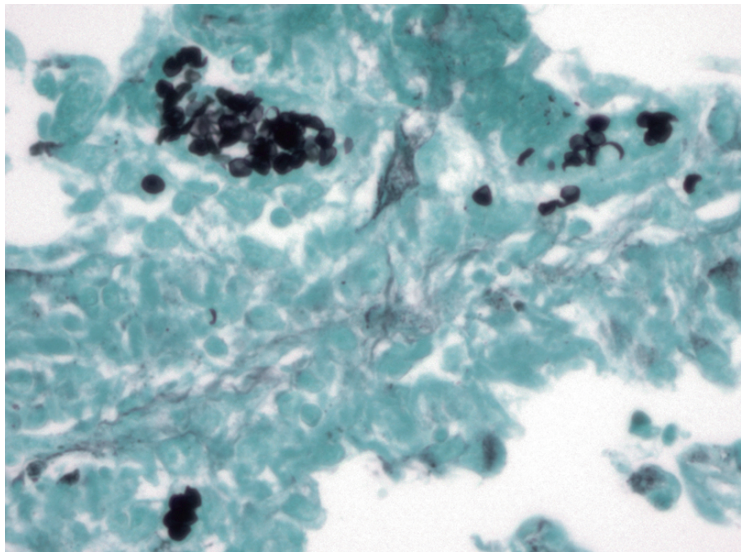

Figure 3.16. *Pneumocystis*. This photograph in the lung is taken at the same power as Figures 3.14 and 3.15. The organisms are stained with Gomori's methenamine silver stain.

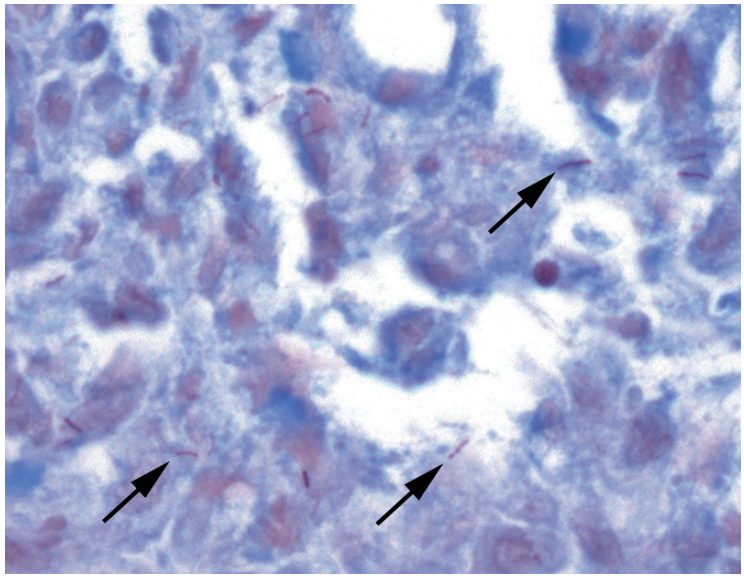

Figure 3.17. Mycobacteria on acid-fast bacteria stain. In this example, tiny wine-red rods are visible within the tissue (arrows).

Viruses

Herpes simplex virus tends to cause extensive tissue damage and ulcers. It infects the epithelium, so look in the cells immediately adjacent to the ulcer. The cells become multinucleated, with the transformed nuclei molding into each other. The chromatin is entirely displaced by glassy nuclear inclusions (viral proteins), outlined by a dark rim of residual chromatin, as though the nucleus is being digested from the inside (Figure 3.20).

Cytomegalovirus can also cause ulcers but may infect tissue without obvious localizing damage. It infects epithelial, endothelial, and mesenchymal cells. In the case of an ulcer, look

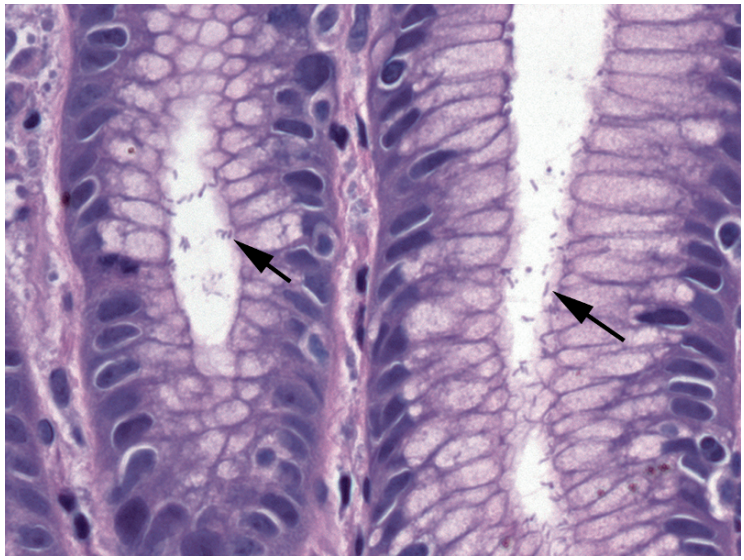

Figure 3.18. *Helicobacter pylori*. The bacilli are sometimes visible on hematoxylin and eosin stain, as seen here (arrows), in the pits of the gastric mucosa.

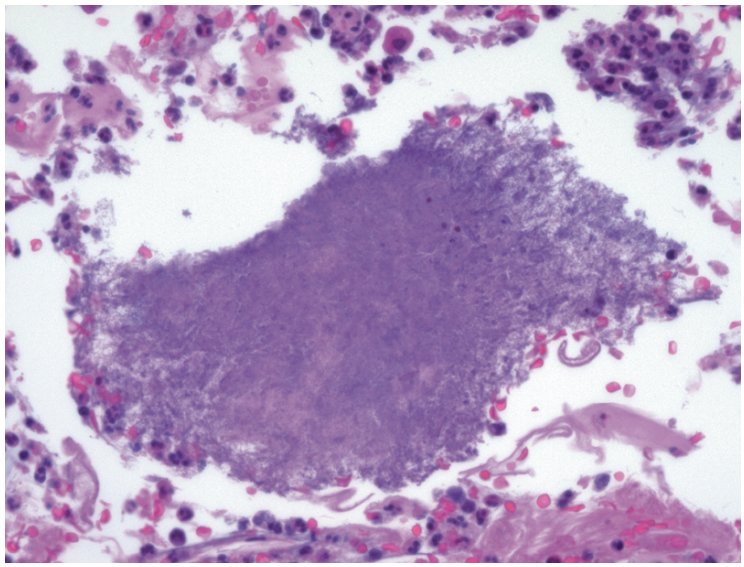

Figure 3.19. *Actinomyces*. This filamentous ball of organisms is easily overlooked, as it resembles fibrin.

in the ulcer bed, not the periphery. The virus causes enlarged cells with large nuclei. The nuclei have a very characteristic inclusion; a dark dense round/oval inclusion surrounded by a pale halo, all within the nuclear membrane (Figure 3.21). The pale halo is not always entirely visible, so finding large dark round nuclei in a group of normal cells (nonneoplastic) should prompt you to consider cytomegalovirus. Immunostains help.

Parasites

Giardia is a duodenal parasite that looks a little like a flounder with a long tail: it is kite shaped when viewed from above but a flat crescent from the side. It is found at the luminal surface of the villi and may not cause much inflammation. The parasites look very much like debris, but in a fortuitous cut

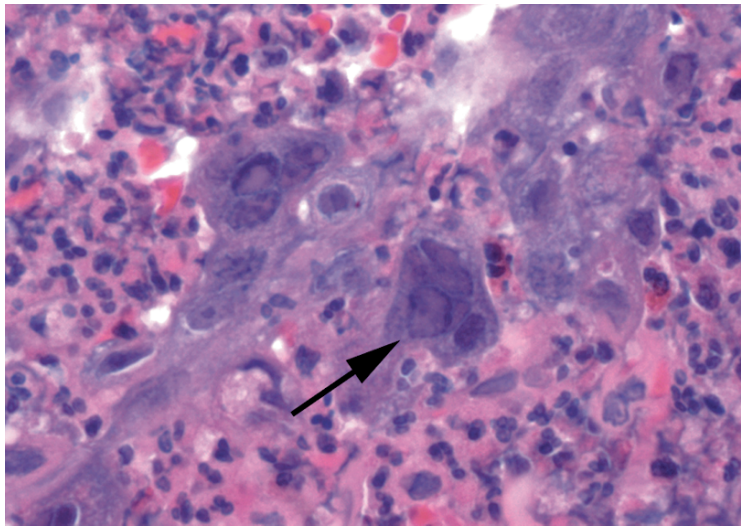

Figure 3.20. Herpesvirus. The classic nuclear changes include multiple molded nuclei with a peripheral rim of chromatin and a glassy inclusion nearly replacing the chromatin (arrow).

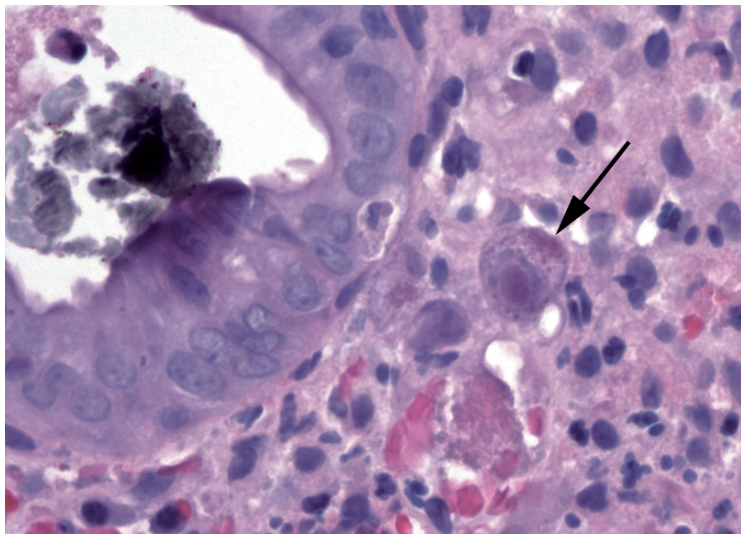

Figure 3.21. Cytomegalovirus. This infected endothelial cell in the gastrointestinal tract (arrow) shows the typical nuclear changes of cytomegalovirus, with a central reddish dense nuclear inclusion, surrounded by a clear halo and a rim of purple chromatin.

you may see the "eyes," which identify it. You will not see *Giardia* unless you look for it. It is related to *Trichomonas*, which you will see on pap smears.

Cryptosporidium is another duodenal parasite that mainly infects the immunocompromised. The tiny round parasites line up along the brush border like clinging bubbles.