Chapter 2
Normal Components

Epithelial Elements

Tracheal and Bronchial Respiratory Epithelium

Normal bronchial respiratory epithelium usually appears as monolayer tissue fragments and strips in bronchoscopic brush, lavage, aspirate, or transbronchial/tracheal fine needle aspirations. Epithelial cells have a uniform, honeycomb appearance *en face* and columnar shape with basally located round uniform nuclei, terminal plates, and cilia from profile (Fig. 2.1). Rare goblet cells (Fig. 2.2) and reserve cells (Fig. 2.3) can be present; they are more frequently found in reactive conditions (see Chap. 3).

Alveolar Epithelium

Normal type 1 pneumocytes are not recognized in cytologic specimens. Type 2 pneumocytes are present in bronchoalveolar lavages (BAL) and fine needle aspirations and may resemble histiocytes, but they have denser cytoplasm and lack phagocytized material (Fig. 2.4). The nuclei of type 2 pneumocytes stain positively for TTF-1, while the cytoplasm is immunoreactive to Napsin A in an intense granular pattern. Pulmonary macrophages may have Napsin A positive phagocytized material in their cytoplasm.

Y.S. Erozan and I. Ramzy, *Pulmonary Cytopathology*, Essentials in Cytopathology 15, DOI 10.1007/978-1-4899-7396-2_2,

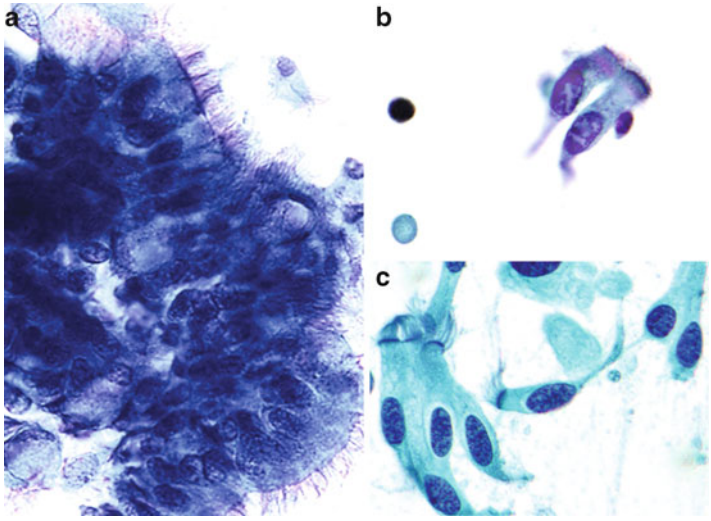

FIG. 2.1. Respiratory epithelium. (**a**) A sheet of ciliated columnar cells. (**b**) Two cells with terminal bars and cilia. (**c**) The oval nuclei have finely granular open chromatin and small nucleoli (Papanicolaou, high power).

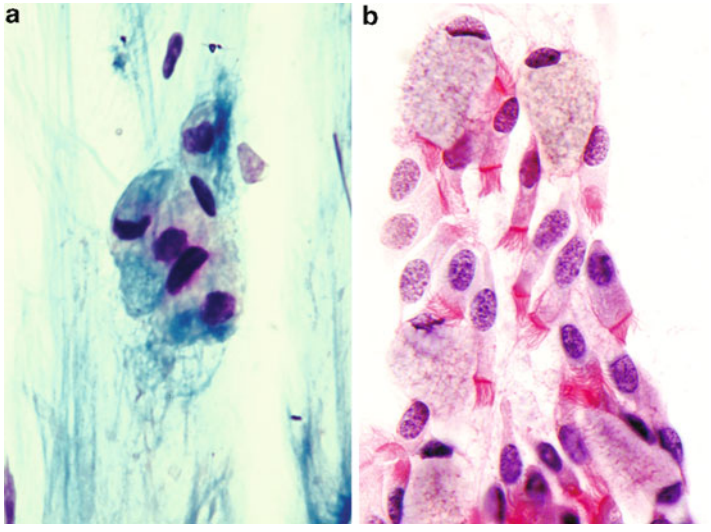

FIG. 2.2. Respiratory mucinous cells. (**a**) Columnar nonciliated cells in mucinous background, sputum. (**b**) Mucinous cells mixed with ciliated cells, Bronchial brush (Papanicolaou, high power).

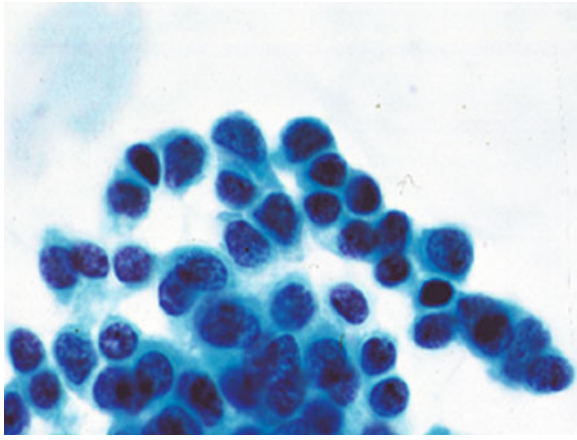

FIG. 2.3. Reserve cells with scant cyanophilic cytoplasm and small hyperchromatic nuclei. These cells are not usually encountered in bronchial material under normal conditions (Papanicolaou, oil, ×100 objective).

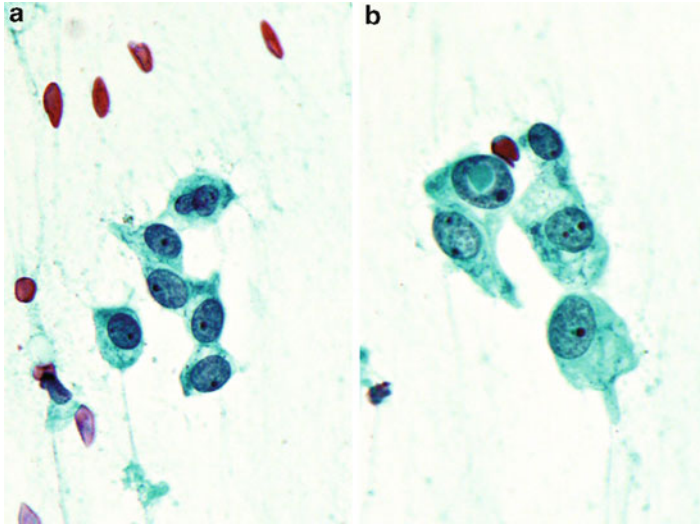

FIG. 2.4. Alveolar epithelium of type 2 pneumocytes showing in (**a**) cells with moderate amount of cytoplasm that lacks phagocytized material, unlike macrophages. (**b**) Some nuclei may have intranuclear cytoplasmic vacuoles (Papanicolaou, oil, ×100 objective).

Nonepithelial Cellular Elements

Specimens procured from normal individuals contain a variety of nonepithelial inflammatory cells including macrophages, eosinophils, neutrophils, and lymphocytes. The number and type of cells are influenced by the method of sampling, by processing, and by the presence of history of smoking. Although it is not practical to have a differential cell count, some general assessment is usually possible. Smokers, particularly those with chronic bronchitis, have larger numbers of inflammatory cells in their BAL specimens with predominance of pulmonary macrophages and neutrophils, as compared to nonsmokers. The significance of finding these cells depends on the cell type, numbers, distribution, and associated lesions. They may represent reaction to injury, to a nearby neoplasm, or to a manifestation of a systemic process.

Macrophages and Giant Cells

These are common elements in pulmonary specimens, particularly in BAL material where they account for 60–90 % of the cells (Fig. 2.5). Increased numbers of macrophages are usually associated with inflammatory conditions such as pneumonia, granulomas, or bronchitis. However, they can also be seen in close proximity to malignant tumors, particularly when there is extensive necrosis. The cells vary in size and have bland oval or kidney-shaped nuclei with finely granular chromatin and small nucleoli. Macrophages are often multinucleated; the nuclei within each multinucleated cell are of similar size and morphology (Fig. 2.6). Rarely do these cells raise differential diagnostic challenge, in view of the evenly distributed fine chromatin and uniformly thin nuclear membrane. Reactive macrophages, however, may have large nuclei with prominent nucleoli and cytoplasmic vacuoles, raising the possibility of adenocarcinoma. The presence of a spectrum that encompasses normal and atypical macrophages with similar nuclear shapes and chromatin characteristics speaks against adenocarcinoma.

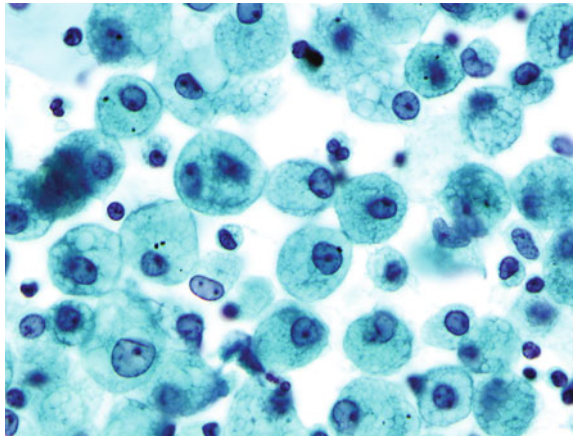

FIG. 2.5. Pulmonary macrophages with vesicular nuclei and foamy cytoplasm (Papanicolaou, oil, ×100 objective).

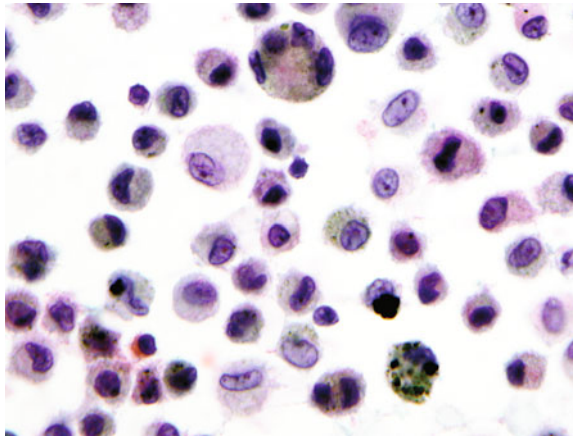

FIG. 2.6. Macrophages showing occasional multinucleation (Papanicolaou, high power).

Several extrinsic and extrinsic elements can be seen within the cytoplasm of macrophages (Fig. 2.7). Examples of intrinsic elements are hemosiderin, lipid, lipofuscin, and blood cells. The tan brown granules seen in smokers stain positively with iron,

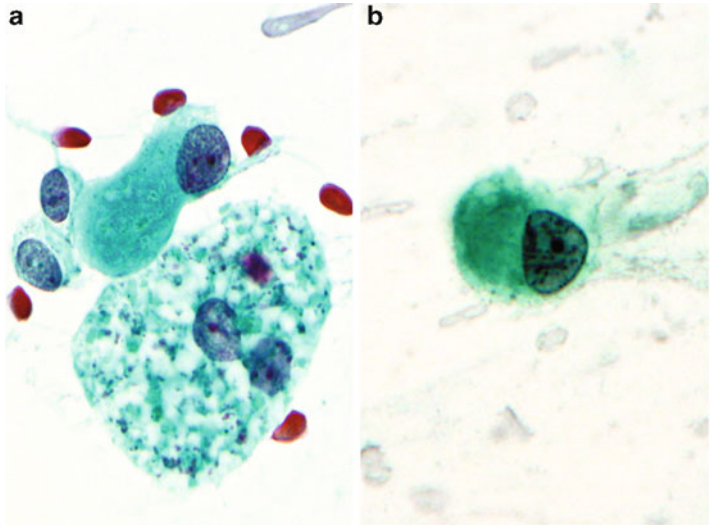

FIG. 2.7. Pulmonary macrophages with phagocytized material in (**a**). The cell in (**b**) shows dense cytoplasm and an eccentric nucleus (Papanicolaou, oil, ×100 objective).

but the granules are finer and stain less intensely than those of siderophages. Although the presence of phagocytized lipid may indicate lipid pneumonia, it can also be encountered in idiopathic pulmonary fibrosis, bronchiectasis, and obstructive conditions. Extrinsic elements that may be phagocytized by macrophages include carbon particles, silica and asbestos fibers. Biologic agents such as *Mycobacterium tuberculosis* or fungi produce a reaction that often includes multinucleated giant cells and is characteristic, though not pathognomonic, of the causative organism. The histiocytic response can be altered in immunocompromised patients, as in the case of tuberculosis when Langhans giant cells are lacking. Identification of some organisms may be feasible on the basis of their morphology, such as in the case of coccidioidomycosis illustrated in Fig. 2.8; other organisms often require microbiologic studies of the pulmonary sample.

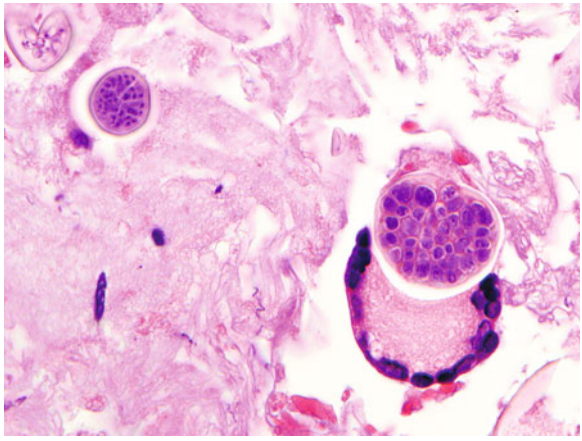

FIG. 2.8. Giant cell and necrosis: This giant cell reaction is associated with coccidioidomycosis infection (H & E, medium power).

Multinucleated giant cells, other than histiocytes, can be seen in a variety of lesions, including sarcoidosis, giant cell interstitial, and viral pneumonias, especially in bronchial brushings. The cytologic features of giant cell interstitial pneumonia include large numbers of multinucleated giant cells and nonpigmented macrophages. This rare condition is associated with occupational exposure to heavy metals. Giant cells associated with viral infections are discussed later.

Siderophages

The cytoplasm of these macrophages contains golden-brown hemosiderin granules that are partially refractile, unlike the dusky blue-black carbon pigment seen in anthracosis (Fig. 2.9). Siderophages are seen in patients with congestive heart failure and infarcts and in cases of hemorrhage associated with Goodpasture syndrome, Wegener granulomatosis, and idiopathic pulmonary hemosiderosis. Positive staining with Prussian blue confirms the nature of the pigment and differentiates hemosiderin from lipofuscin and melanin.

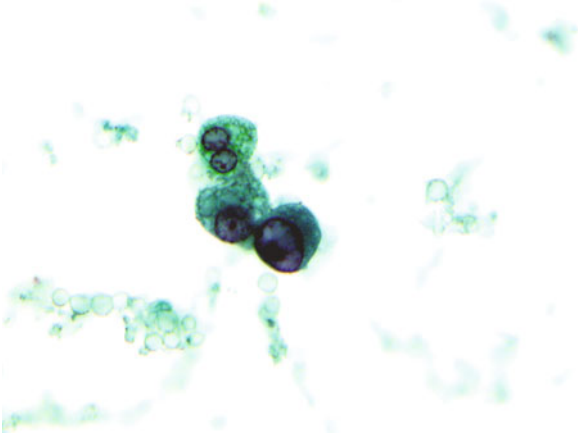

FIG. 2.9. Siderophages with opaque pigment granules that vary in size. These would stain positively with Prussian blue and negatively with melanin immunostains, such as Melan A (Papaniolaou, oil, ×100 objective).

Polymorphonuclear Leukocytes

A wide variety of conditions are associated with the presence of neutrophils in pulmonary specimens, other than contaminants from oral contents. The cells are seen in large numbers in acute bronchitis, bacterial pneumonias, and abscesses. They are also encountered in BAL specimens procured from smokers and from patients with asbestosis, idiopathic pulmonary fibrosis, scleroderma, rheumatoid arthritis, diffuse alveolar damage, adult respiratory distress syndrome, and others. Acute inflammatory cell infiltrate is also frequently present as a component of the necrotic background associated with malignant neoplasms.

Lymphocytes

Small mature lymphocytes are not uncommon in specimens procured by bronchial brushings, bronchial lavage, or bronchioloalveolar lavage. An abundance of lymphocytes may be encountered in transbronchial Wang needle aspirates and is an

indication of adequate sampling of the hilar lymph nodes. In BAL specimens, most of the lymphocytes encountered are of T cell lineage, with a helper to suppressor ratio of 1:8. Such ratio is altered in favor of helper (CD4 positive) cells in sarcoidosis or towards suppressor (CD 8 positive) cells in hypersensitivity pneumonitis. Granulomatous and viral infections, hypersensitivity, and drug-induced pneumonitis are associated with increased number of lymphocytes. In follicular bronchitis, large immature lymphocytes and tingible body macrophages are seen in addition to mature lymphocytes.

Conditions associated with abundant lymphocytes

- Granulomas (tuberculosis, sarcoidosis)
- Hypersensitivity pneumonitis
- Drug-induced reactions
- Viral infections
- Lymphoma/leukemia

Differential Diagnosis

Chronic inflammatory lesions may present as a mass, raising the possibility of a neoplasm. Conversely, a neoplasm may result in bronchial obstruction and subsequent chronic inflammation. In the presence of abundant lymphocytes, small cell carcinoma and lymphoma should be considered in the differential diagnosis. The nuclei of neoplastic cells of small cell carcinoma are larger than those of mature lymphocytes, with pleomorphism, molding, and streaking of chromatin. Immunostains for neuroendocrine markers are usually needed for differentiating the two cell types. In cases of pulmonary involvement by lymphoma or leukemia, the lymphocytic infiltrate is more prominent; adequate clinical data and submitting an aliquot for flow cytometric analysis are critical in establishing the correct diagnosis.

Eosinophils

These inflammatory cells account only for 1 % of cells in BAL material in normal individuals. They are usually seen in response

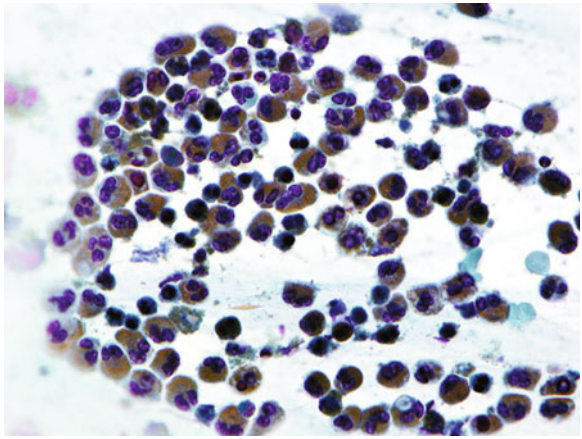

FIG. 2.10. Eosinophils in large numbers in sputum from a patient with allergic pneumonitis of undetermined etiology (Papanicolaou, medium power).

to antigenic stimulation, such as in bronchial asthma, parasitic and fungal infections, hypersensitivity pneumonitis, and eosinophilic pneumonia. On Papanicolaou stained material, eosinophils do not exhibit the bright red or pink granules seen with Romanowsky stains; instead, the granules are somewhat yellowish green and refractile, and the cells are easily recognized by their characteristic bilobed nuclei (Fig. 2.10). Abundant eosinophils are associated with rhomboid Charcot–Leyden crystals, which are described later.

Conditions associated with abundant eosinophils

- Hypersensitivity pneumonitis
- Drug-induced reactions
- Parasitic infestations
- Bronchial asthma
- Eosinophilic pneumonia

Megakaryocytes

Though often seen in alveolar septa, megakaryocytes are rarely encountered in respiratory cytologic specimens. They are characterized by multiple nuclei that are centrally located. The nuclei are hyper-

chromatic and pleomorphic, unlike the uniform vesicular nuclei of histiocytic giant cells.

Miscellaneous Noncellular Elements

Curschmann Spirals

These are mucous casts of medium-sized to small bronchioles that are commonly found in sputa of heavy smokers. They can also be encountered in material procured from patients with bronchial asthma, chronic bronchitis, and other obstructive lung disease. They form corkscrew like spirals; each has a central core from which filamentous structures radiate perpendicular to the longitudinal axis of the core. Curschmann spirals stain pale cyanophilic or eosinophilic with Papanicolaou and black with silver stains (Fig. 2.11).

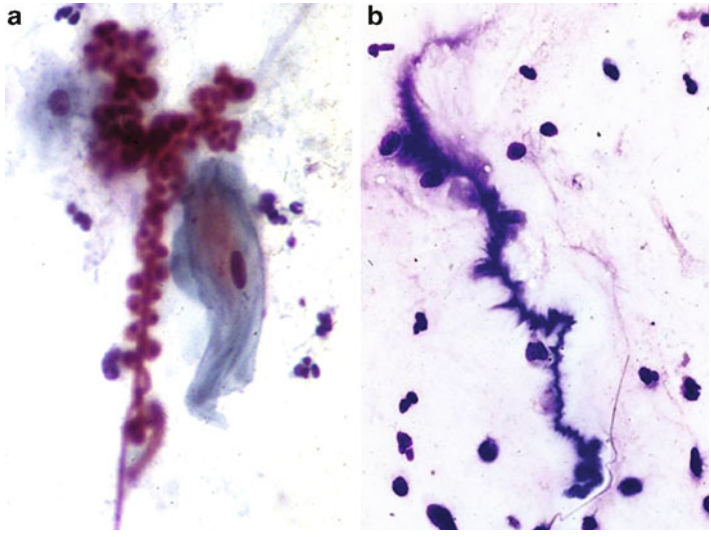

FIG. 2.11. Curschmann spiral. The inspissated mucus is a cast of bronchial lumen (**a** and **b** Papanicolaou and Diff-Quik respectively, medium power). (**a**) from Ostrowski MO, Ramzy I, *In: Ramzy I: Clinical Cytopathology & Aspiration Biopsy, ed 2,* New York, NY, McGraw-Hill, 2001, by permission.

Charcot–Leyden Crystals

These crystals form as a result of condensation of the cytoplasmic granules of eosinophils. These are rhomboidal or needle-shaped structures of variable sizes and stain green or red on Papanicolaou stained material, with sharply defined and refractile edges (Fig. 2.12). Charcot–Leyden crystals can be observed in many conditions in which there is increased number of eosinophils, such as in bronchial asthma or allergic pneumonitis due to different causes.

Blue Bodies (Corpora Amylacea)

Corpora amylacea are globular structures that form within alveolar spaces and are more likely to be associated with pulmonary edema, infarction, or chronic bronchitis. The acellular bodies are large spherical, with a central birefringent core that is surrounded by homogeneous or lamellated material (Fig. 2.13). Although concentric laminations may be seen, they do not calcify similar to psammoma bodies. Identification of corpora amylacea has no clinical significance.

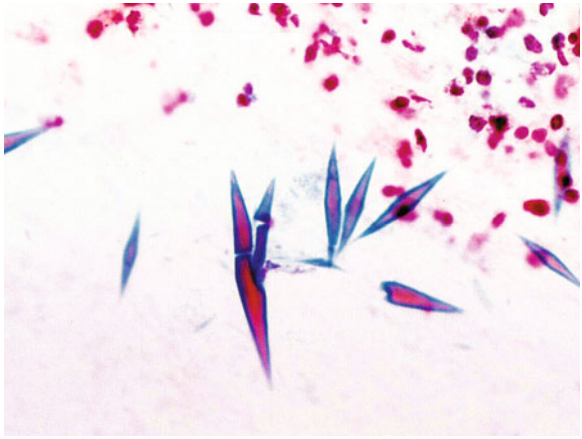

FIG. 2.12. Charcot–Leyden crystals. Sputum from a patient with bronchial asthma. The rhomboid crystals vary in size and stain *greenish or red* with Papanicolaou stain (Papanicolaou, medium power).

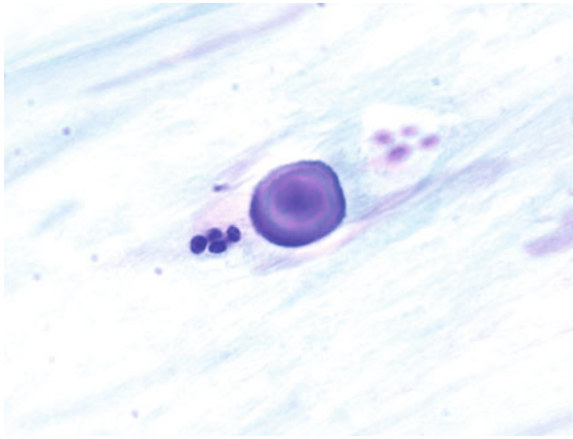

FIG. 2.13. Corpora amylacea. The pale cyanophilic structures may show faint lamination but are not calcified (Papanicolaou, high power).

Calcific and Psammoma Bodies

Calcified irregularly shaped structures can be encountered in chronic granulomas, such as tuberculosis, and are nonspecific. Well-organized lamellated calcified psammoma bodies may be seen in a variety of primary or metastatic papillary neoplasms. These include bronchioloalveolar carcinoma, metastatic thyroid carcinoma, and ovarian carcinoma (Fig. 2.14). The presence of psammoma bodies should not be taken as conclusive evidence of malignancy in the absence of epithelial cells with clear nuclear features of malignancy. Other lesions, including the rare benign condition of alveolar microlithiasis, may be associated with psammoma bodies.

Contaminants

A wide variety of food particles, such as vegetable material and meat fibers, as well as bacterial colonies, pollen, and other debris may be encountered in specimens arriving to the laboratory (Fig. 2.15). Some of these may superficially resemble fungal organ-

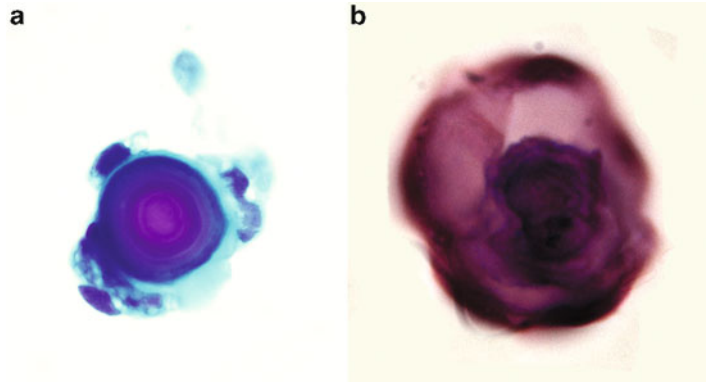

FIG. 2.14. Psammoma bodies. (**a** and **b**) The lamellated calcified structures can be encountered in benign and malignant lesions (Papanicolaou, high power).

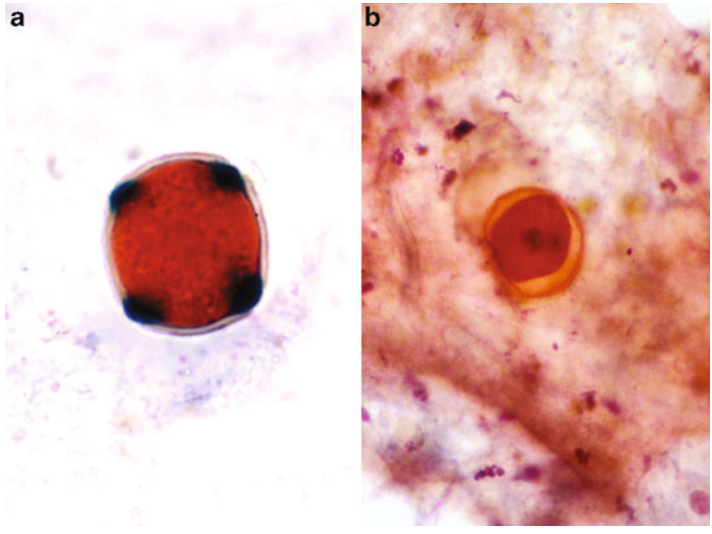

FIG. 2.15. Plant cells. (**a**) Pollens and (**b**) plant cells. Note characteristic refractile cell walls and often geometric patterns that easily distinguish these from keratinized squamous cells (Papanicolaou, high power).

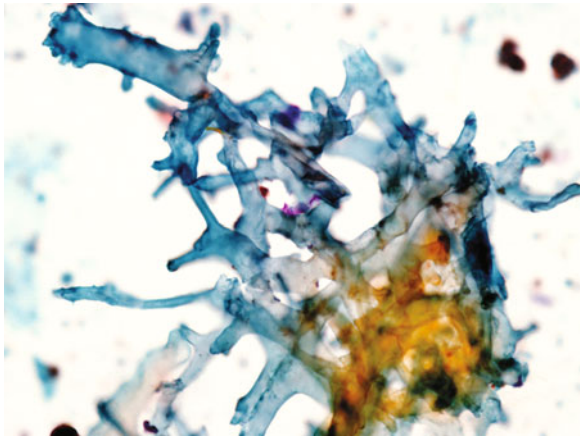

FIG. 2.16. Vegetable material. These fibers may be encountered as a result of oral contamination of the specimen. Their irregular diameters help differentiating them from zygomycetes (Papanicolaou, medium power).

isms or even squamous cells, but establishing their nature as oral contaminants does not present a problem in most cases (Fig. 2.16). However, the significance of finding bacteria or fungi may not be clear in some cases, and clinical correlation with radiologic correlation is necessary. Presence of food particles is rarely an indication of pulmonary aspiration.

Suggested Reading

Antonakopoulos GN, Lambrinaki E, Kyrkou KA. Curschmann's spirals in sputum: histochemical evidence of bronchial gland ductal origin. Diagn Cytopathol. 1987;3:291–4.

Chen KTK. Psammoma bodies in fine-needle aspiration cytology of papillary adenocarcinoma of the lung. Diagn Cytopathol. 1990;6:235–42.

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