TWO

Pathologic Diagnosis of Pulmonary Disease

Pathology of Pulmonary Disease, edited by Mario J. Saldana.
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Cytologic Examination and Fine Needle Aspiration

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The remarkable progress in pulmonary cytopathology during the past three decades has largely resulted from advances in the methods of specimen collection, which allow virtually any portion of the bronchial tree, lung parenchyma, and pleura to be reached and sampled. Many benign diseases are becoming diagnosable by cytologic means, including inflammatory, infectious, degenerative, environmental, and neoplastic disorders of the lung. The increased sensitivity and specificity of cytopathology in the early diagnosis of pulmonary malignancy has been matched by improvements in the staging of lung cancer. In this chapter, the main methodologic and interpretative aspects of pulmonary cytology are reviewed.

TECHNICAL ASPECTS

Respiratory specimens suitable for cytologic examination are presented in Table 3-1. Each technique offers certain advantages and disadvantages that should influence a particular choice.

Sputum production is common in respiratory infections, and caution should be exercised in the handling of these specimens in the cytology laboratory. This is also the case for bronchoalveolar lavage (BAL), which is often used in the diagnosis of opportunistic infections.[1–4] As a rule, all respiratory specimens should be considered contaminated and handled under a ventilated hood.

Fine needle aspiration (FNA) is suitable for elucidating localized lesions (*e.g.*, nodules, masses, localized infiltrates), its primary target being a lesion suspected of malignancy. **Imprint smears** are useful in the rapid diagnosis of opportunistic pulmonary infections and tumors. Pulmonary microvascular cytology is performed with the use of a pulmonary artery catheter. This technique has been used in the evaluation of lymphangitic carcinomatosis, amniotic fluid, and fat embolism.[5–7]

Crucial to any of the techniques in Table 3-1 is rapid fixation of the collected specimen, appropriate cytopreparatory techniques, and good staining (Table 3-2). Sputum specimens should be collected directly into a fixative bottle and processed according to preference and the availability of resources. The pick-and-smear technique demands less time and ensures representative sampling while maintaining the usual relations among the exfoliate cells. The Saccomanno homogenization method is recommended for the screening of specimens with metaplastic and other preneoplastic changes.[8]

We recommend that the bronchoscopist be assisted by a cytotechnologist in the preparation of slides from flexible brushes. FNA should be collected with the assistance of cytopathology personnel to ensure good cellularity. In our experience, aspirates collected in Plasmalyte (Travenol, Deerfield, IL) allow multimodal assessment of the specimen, including special stains, electron microscopy, and immunocytochemistry.

NORMAL CELL POPULATION OF THE RESPIRATORY TRACT

Five major epithelial cell types are significant in clinical cytologic samples from the respiratory tract: ciliated cells, goblet cells, reserve cells, Kultchitsky-like cells, and pneumocytes. Nonepithelial cells, such as macrophages and megakaryocytes, are also observed with fibroblasts and other mesenchymal elements (*e.g.*, lymphocytes, eosinophils); mesothelial cells may be found in FNA.

Ciliated Cells

Ciliated columnar cells are numerous in pulmonary samples because of their wide distribution from the nose down to the level of the bronchioles. These cells have centrally or basally placed oval nuclei, one tapered end, and prominent terminal plates to which

TABLE 3-1
Comparison of Various Pulmonary Cytologic Procedures

Specimen	Advantage	Disadvantage
Sputum	Suitable for screening patient population with high risk for lung cancer	Patients not always instructed properly on how to cough
Bronchial brushings	Cellular samples from extensive portions of bronchial tree	Blood and inflammatory debris may obscure significant cells
Bronchial washings	Cells may be obtained from very peripheral lesions	Only limited portion of bronchial tree is sampled
Bronchoalveolar lavage	Ideal for evaluation of interstitial lung diseases and opportunistic infections	Available only in a few specialized centers
Fine needle aspiration	Evaluation of any portion of the lung by a semi-invasive approach	Bleeding and pneumothorax are possible complications
Imprint	Rapid diagnosis and excellent cytologic detail, including unusual infection	Invasive procedure that requires thoracotomy
Microvascular sample	Evaluation of intravascular lesions like fat emboli, amniotic fluid embolus, and lymphangitic carcinomatosis	Pulmonary artery catheterization is required

Adapted from Bedrossian CWM, Accetta PA, Kelly LV. Cytopathology of nonneoplastic pulmonary disease. Lab Med 1983;14:86.

TABLE 3-2
Cytopreparatory Methods for Pulmonary Specimens

Collection Technique	Slide Preparation	Staining Procedure
SPUTUM		
70% alcohol in glass jar or Saccomano fluid in mailable container	Wet film prepared by pick-and-smear technique or homogenization in blender	Papanicolaou and Gomori methenamine silver stains in smears
BRUSHES		
Glass slides immersed in Coplin jars with 95% alcohol	Slides prepared by endoscopist with assistance of laboratory personnel	Papanicolaou and Gomori methenamine silver stains in smears
WASHINGS		
80% alcohol added to bronchoscope trap: postfixation of cell button in B-5 fixative	Cell blocks and smears prepared from centrifugate or membrane filtration (*i.e.*, Gelman)	Papanicolaou and Gomori methenamine silver stains for smears; hematoxylin & eosin and special stains for cell blocks
NEEDLE ASPIRATES		
Collect and transport in Plasmalyte; transfer to 80% alcohol for light microscopy and 3% glutaraldehyde for electron microscopy	Slides prepared by laboratory personnel in procedure room; smears and membrane filters prepared in laboratory	Fast and routine Papanicolaou stains; uranyl acetate–lead citrate stains for electron microscopy

Cilia are attached. Ciliated cells may shed individually, in a mosaic pattern, or in palisading fashion with nuclei at different heights, simulating multinucleation (Fig. 3-1). They may also occur in the form of cell balls, distinguishable from true malignant cells by the presence of cilia or terminal plates. The cilia are resistant to degeneration, and their loss from intact columnar cells bearing terminal plates indicates irritation of the epithelium. In injuries induced by viruses and other agents, cilia may remain attached to pinched-off portions of cytoplasm, a phenomenon known as ciliocytophtoria (Fig 3-2).[9]

Goblet Cells

Mucus-secreting cells of the bronchial tree are sparsely distributed throughout the bronchial mucosa but do not extend to the bronchioli epithelium except in irritative conditions. Goblet cells respond readily to injury and proliferate in the surface epithelium and in the submucosal bronchial glands. They are responsible for increased production of mucus, which indicates chronic bronchitis.[10] In cytologic specimens, goblet cells degenerate rapidly because they are rich in hydrolytic enzymes. The presence of even a few of these cells indicates mucus hypersecretion. Goblet cells appear as rounded or elongated elements with relatively small nuclei, pushed aside by a clear cytoplasm filled with mucus (Fig. 3-3). Goblet cells often occur in close contact with columnar cells as part of cell balls known as Creola bodies. These appear atypical and should not be confused with malignancy (Fig. 3-4).

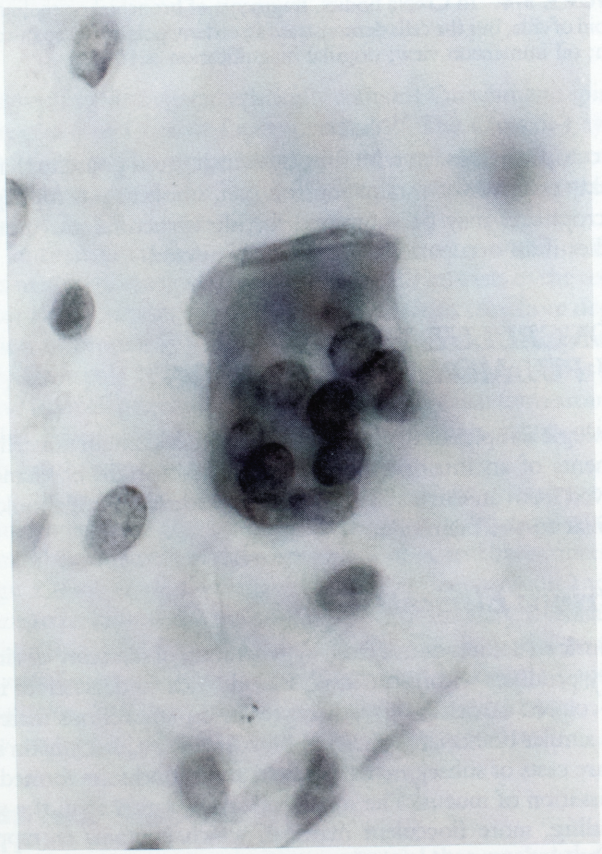

FIGURE 3-1. A multinucleated ciliated cell contains bland nuclei located at different heights. (Papanicolaou stain; oil immersion view; original magnification ×1000.)

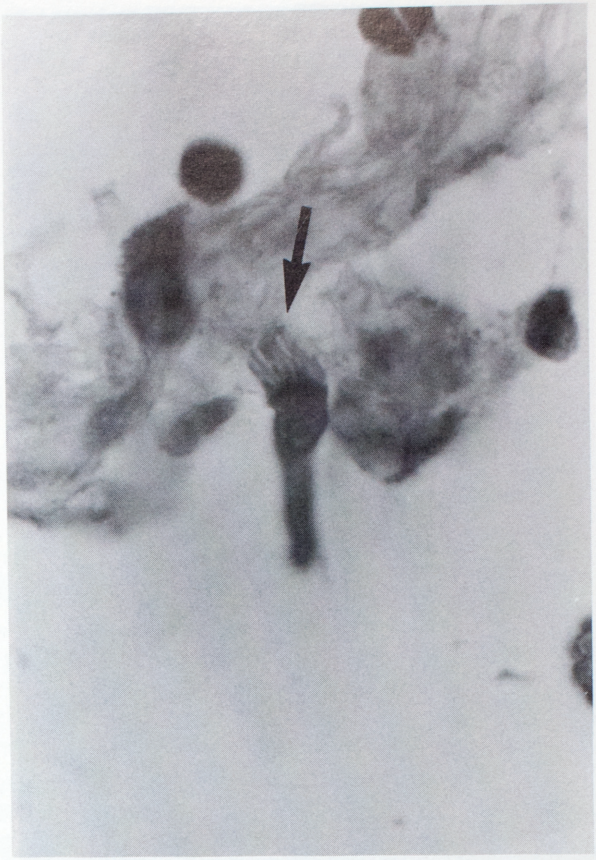

FIGURE 3-2. In ciliocytophtoria, a fragment of cytoplasm lacking a nucleus displays intact cilia and a terminal plate (*arrow*). (Papanicolaou stain; oil immersion view; original magnification ×1000.)

Kultchitsky-like Cells

Also referred to as K cells or Freyter cells, Kulchitsky-like cells have neuroendocrine granules easily demonstrable by immunoperoxidase (*i.e.*, chromogranin) and by electron microscopy. They are triangular and located in the basal portion of the mucosa. These cells are positively stained with silver stains and are thought to be the cell of origin of neuroendocrine tumors like carcinoid, atypical carcinoid, and small cell carcinoma. Kultchitsky-like cells are not neural crest in origin but derived from ordinary precursor cells of the bronchial mucosa.

Reserve Cells

Basal cells are believed by many to represent the reserve cells of the bronchial mucosa. However, McDowell and colleagues suggested that immature, small mucous granular cells are the true precursors of basal cells and other bronchial epithelial cells, including Kultchitsky-like cells.[11] Basal cells are more commonly seen in bronchial brushings. They exfoliate in clusters or sheets with regular sizes and shapes and often have hyperchromatic nuclei that may cause concern in assessing poorly preserved specimens. Differentiation of reserve cells from hyperplastic or neoplastic cells may be difficult at times.[12]

Immature, small mucous granular cells are seldom recognized in cytologic specimens. Pending confirmatory cytopathologic and ultrastructural correlative studies, these cells may represent the groups of cuboidal cells with vacuolated cytoplasm that sometimes

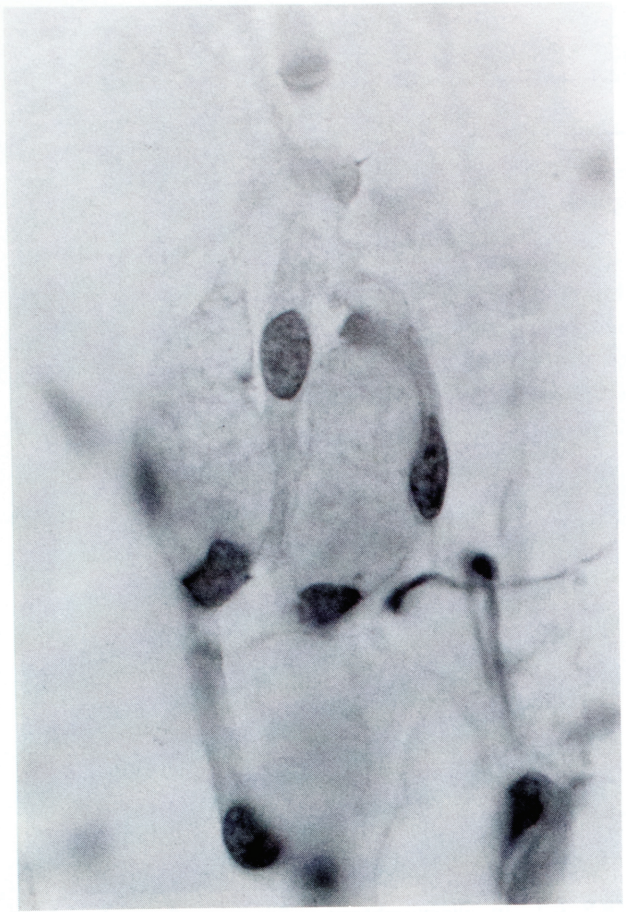

FIGURE 3-3. In goblet cells, the mucus-filled cytoplasm pushes the nuclei toward the base. (Papanicolaou stain; oil immersion view; original magnification ×1000.)

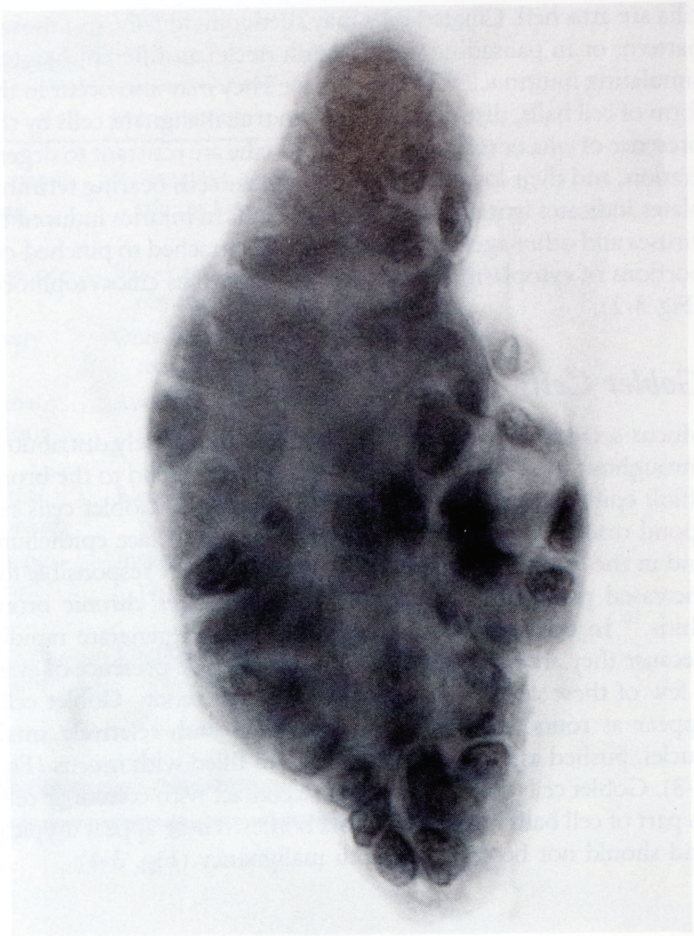

FIGURE 3-4. In Creola bodies, fragments of bronchial epithelium are devoid of cilia, but the cells demonstrate an orderly polarity. (Papanicolaou stain; oil immersion view; original magnification ×1000.)

exfoliate in sheets but lack the degree of keratinization that differentiates metaplastic cells. When identified, small mucous granular cells may be referred to as immature metaplastic cells or columnar cells that have lost cilia. These cells are probably totipotential and play an important role in proliferative responses.

Alveolar Pneumocytes and Macrophages

The alveoli are lined by type I (*i.e.*, squamous) pneumocytes and type II (*i.e.*, granular) pneumocytes. The type II pneumocytes are involved in the production of surfactant and are capable of proliferation in response to injury.[13] They may become reactive and atypical, or they may be confused with alveolar macrophages.

Approximately two thirds of macrophages derive from the bone marrow and one third from tissue histiocytes of the lung interstitium.[14] They are the *sine qua non* for a satisfactory sputum specimen, such as one from a deep cough. The distinguishing feature of alveolar macrophages is dust or pigment in the abundant cytoplasm, which otherwise may appear granular or contain a variety of inclusions.

Cells of histiocytic origin may form giant cells with centrally or peripherally located nuclei (Fig. 3-5). In patients with inorganic dust exposure, the macrophages contain numerous cytoplasmic particles, the extent of which depends on the occupational exposure.[15,16] Alveolar macrophages may also reveal whether the patient is a smoker. In nonsmokers without exposure to inorganic dust, macrophages have few cytoplasmic particles. In heavy smokers, the cytoplasm is laden with fine, brownish green granules that are weakly positive with stains for iron (*i.e.*, smoker's macrophages). Macrophages may be submitted for ultrastructural and analytic studies if an occupational disease is suspected.

NONCELLULAR ELEMENTS IN PULMONARY CYTOLOGY

Cytologic samples of the respiratory tract may contain noncellular elements of an intrinsic, often degenerative origin or elements derived from an extrinsic, environmental source. Both should be familiar to the examiner.

Intrinsic Elements

Intrinsic structures derive from degeneration of secretory or circulatory products within the lung. Blood tends to degenerate into rust-colored carcasses of erythrocytes or an amorphous material with similar tinctorial properties. Mucostasis or Curschmann spirals are casts of subsegmental bronchi and bronchioles formed by inspissation of mucus. The inner coil stains darker than the surrounding, more flocculent material, which contains entrapped cellular elements such as inflammatory or tumor cells (Color Fig. 3-1). Curschmann spirals are associated with overproduction of mucus, as in asthma and chronic bronchitis. The sputum of pa-

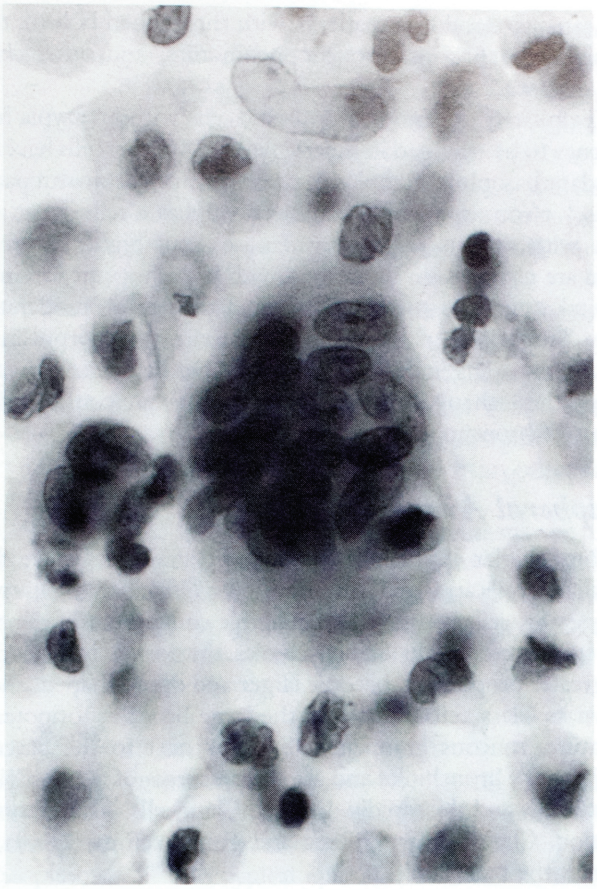

FIGURE 3-5. In a multinucleated giant cell, overlapping nuclei still show their histiocytic derivation. (Papanicolaou stain; oil immersion view; original magnification ×1000.)

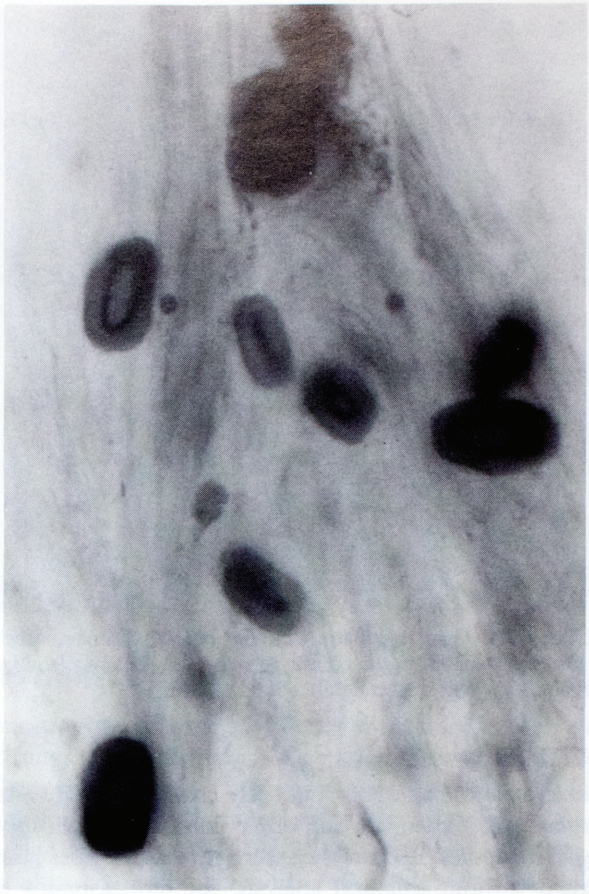

FIGURE 3-6. Multiple calcospherites. The small concretions have a laminated appearance. (Papanicolaou stain; oil immersion view; original magnification ×1000.)

tients with asthma characteristically contains Curschmann spirals, eosinophils, or Charcot-Leyden crystals.[17] The Charcot-Leyden crystals are needle shaped birefringent structures best seen in wet preparations (Color Fig. 3-2). They result from degenerated eosinophils.

In bronchiectasis and mucoid impaction of bronchi, erythrocytes may form elongated, sometimes branched casts of the terminal airways. Corpora amylacea, which appear to arise from degeneration of bronchial secretions, are concretions of glycoprotein. They stain pale yellow with Papanicolaou stain and are usually found in small groups. Corpora amylacea may also form around a nucleus represented by plant spores, and are commonly associated with heart failure and pulmonary infarction.

Calcospherites are cyanophilic concretions containing calcium, phosphates and other minerals, such as iron and magnesium. They are smaller than corpora amylacea, and have concentric rings. Calcospherites are the result of cell degeneration and are characteristically abundant in idiopathic pulmonary alveolar microlithiasis (Fig. 3-6).[18] Psammoma bodies, a special form of calcospherites, are usually associated with metastatic papillary neoplasms and bronchioloalveolar cell adenocarcinomas. They may be seen in asbestosis and other pneumoconioses.

Extrinsic Elements

Extrinsic structures are exogenous material that reaches the lung by inhalation or aspiration, or they may be contaminants of the assessment process. With the universal use of gloves in the laboratory, starch granules have become the most common contaminant in cytologic preparations. They differ from corpora amylacea in their chemical composition and appear as characteristic Maltese crosses under polarized light.

Pollen grains vary considerably in size and shape and may be difficult to differentiate from fungi. The pollen grains are birefringent but lack any particular crystalline intrastructure. Their presence does not necessarily indicate an allergic disorder, because they usually originate in the mouth; they probably reflect aspiration, particularly when accompanied by heavy inflammation. The same is true of meat particles, easily identifiable by thin cross striations.

Vegetable cells may appear singly or in groups, in which they may mimic degenerating or even neoplastic cells. They are recognized by their odd square or rectangular shapes and by their thick cellulose cell wall (Fig. 3-7).

Cytologic samples are helpful in the diagnosis of environmental pulmonary diseases by the identification of particles and fibers, whether free or within alveolar macrophages. Silica particles are small, needlelike structures commonly associated with carbon and engulfed by macrophages. They can be visualized with polarized light and do not warrant an immediate diagnosis of silicosis without biopsy-proven fibrosis (see Chap. 35).

Ferruginous bodies are elongated fibers coated with a mixture of protein and iron, which is responsible for their characteristic green or rusty brown color.[19] They are easily identified with the use of Prussian blue stain. Ferruginous bodies are generally accom-

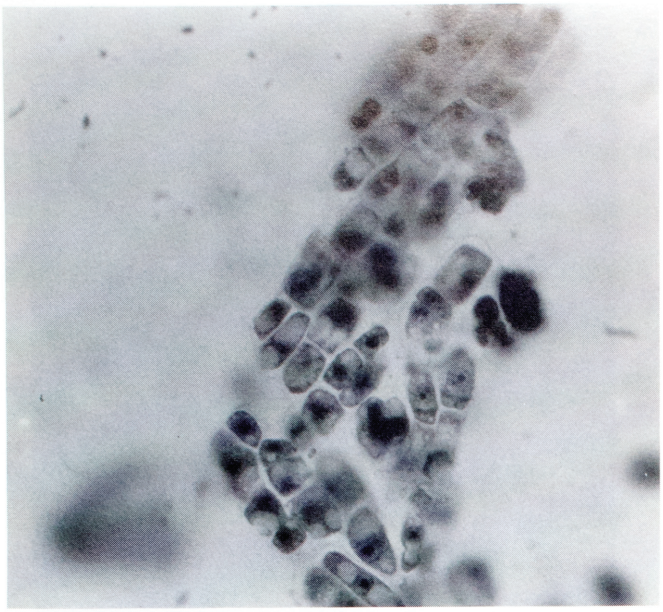

FIGURE 3-7. Vegetable matter contains bipolar vacuoles, which compress nuclei toward the centers of the square cells. (Papanicolaou stain; oil immersion view; original magnification ×1000.)

panied by macrophages. Most of these coated fibers are asbestos, but occasionally fiberglass and other fibrous minerals appear as ferruginous bodies. Because the exact nature of the fiber cannot be discerned without chemical analysis, the term asbestos bodies should not be used to refer to these structures (see Chap. 36).

Blue bodies are birefringent calcium carbonate structures with a blue rim when stained with Papanicolaou stain. They are not significantly associated with pulmonary disease or malignancy (see Chap. 31).[20] Birefringent crystals of calcium oxalate may be the only cytologic manifestation of pulmonary aspergillosis (see Chap. 43).[21] They are commonly associated with an abundant, acute inflammatory exudate.

PRINCIPLES OF CYTOPATHOLOGIC INTERPRETATION

Bronchial Mucosa

Immature small, mucous granular cells are probably the progenitor of basal cells, ciliated cells, and Kultchitsky-like cells, and only a few of them progress to mature mucus-secreting goblet cells.[11,22] This results in a 5 : 1 normal ratio between ciliated and goblet cells in the bronchial epithelium. Because ciliated cells are end-stage cells incapable of division, they do not participate in the epithelial proliferative response. Goblet cells may proliferate under irritative exposure and virtually replace the ciliated epithelium, a phenomenon known as goblet cell hyperplasia, common among cigarette smokers. The presence of goblet cells in the sputum usually reflects chronic bronchitis.

Basal cell hyperplasia can be confused with small cell carcinoma, but the lack of tumor diathesis and necrosis, the cohesiveness of the cells, and the identification of ciliated cells in the cluster point to a benign diagnosis. Basal cell hyperplasia is commonly associated with squamous metaplasia, and it is thought to be its precursor. Both conditions are common in cigarette smokers, but they can also develop in patients with thromboembolism, tuberculosis, cystic fibrosis, bronchopneumonias, and other chronic lung diseases.[23]

Exfoliated squamous metaplastic cells without atypia have a tendency to be arranged in sheets (Fig. 3-8). These cells have fairly abundant basophilic cytoplasm, possess a fine chromatin pattern, and lack pleomorphism. Squamous metaplastic cells occur in patients with various respiratory diseases, but higher degrees of atypia are usually seen in patients with bronchogenic carcinoma. Atypical squamous metaplasia is graded as mild, moderate, or severe. Single cells, nuclear hyperchromasia, pleomorphism, and cytoplasmic orangeophilia indicate a higher degree of atypia. Although these changes can be reversible, they are considered precursors of bronchogenic carcinoma.[14,24–27]

Peripheral Airways

The bronchiolar epithelium is composed mainly of ciliated and nonciliated cells arranged in a single layer. Nonciliated cells resembling serous cells constitute a negligible component of the bronchial epithelium, but in the bronchioles, the nonciliated cells occur in a greater proportion, become larger and dome-shaped, and are known as Clara cells.[28,29] As in the large bronchi, totipotential, immature, mucous granular cells occur next to the basement membrane of bronchioles and are the precursor of the undividing ciliated cells and the rapidly dividing Clara cells. After abnormal irritation, Clara cell hyperplasia and goblet cell metaplasia may occur in the bronchioles.

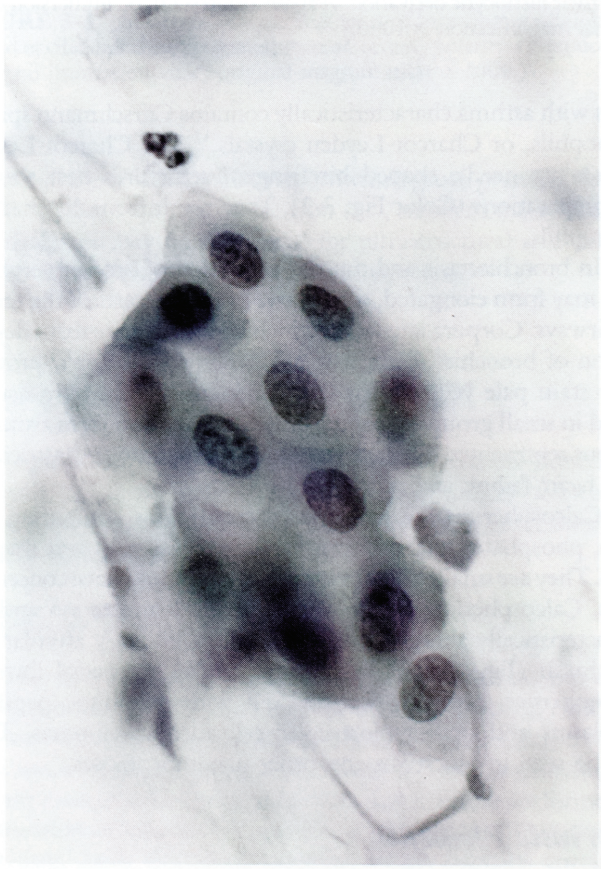

FIGURE 3-8. The sheetlike arrangement and dense cytoplasm of these metaplastic bronchial cells are typical of squamous metaplasia. (Papanicolaou stain; oil immersion view; original magnification ×1000.)

Without the aid of electron microscopy, the histogenesis of the proliferative process cannot be discerned with certainty, and the origin of atypical hyperplastic cells cannot be recognized. These cells may represent the immature mucous cells before they undergo differentiation, and they are often referred to as atypical epithelial or hyperplastic glandular cells. Although the natural history of these cells has not been investigated, they are thought to progress to neoplasia. Because this takes place in the peripheral airways, the commonly resulting tumors are bronchioloalveolar adenocarcinomas, which can be mucinous or nonmucinous (*i.e.*, derived from Clara cells) adenocarcinomas.[30] Pathologic processes producing hyperplasia or neoplasia are often accompanied by fibrosis, as seen in scleroderma lung or in the Hamman-Rich syndrome. Tumors may originate from a localized scar resulting from an old granuloma (*e.g.*, tuberculosis) or from other causes (*e.g.*, healed infarcts; see Chap. 31).

Changes induced by chemotherapeutic agents may be recognized early and may lead to drug withdrawal, avoiding fatal pulmonary toxicity.[31] After radiation therapy, severe epithelial atypia may be confused with malignancy.[32] Knowledge of previous treatment is essential to avoid such pitfalls (see Chaps. 15 and 16).

Air Spaces

Sampling of the air spaces is best achieved with BAL. Examination of this fluid is useful for infectious processes like *Pneumocystis carinii* pneumonia, in interstitial lung disease, and for peripheral neoplasms.[1–3,33,34] Atypical epithelial cells can be found in various diseases. Biyoudi-Vouerze and colleagues described a series of 30 patients whose BAL fluid contained atypical epithelial cells that could indicate malignancy.[35] Carcinoma was identified in only 9 patients, and the remaining patients had other diseases, including idiopathic interstitial fibrosis, pneumoconioses, bacterial pneumonia, non-Hodgkin lymphoma, tuberculosis, lupus erythematosus, hypersensitivity pneumonitis, lipoid pneumonia, and others. The atypical cells encountered in the fluid of these patients had abundant vacuolated cytoplasm, a large nucleus, and a prominent nucleolus. Stanley and associates described similar cells in the BAL fluid of 12 patients with acute respiratory distress syndrome.[13] Amiodarone can cause cytologic atypia with a peculiar vacuolation indicative of phospholipid accumulation in the cytoplasm (see Chap. 16).[36]

CYTOLOGIC DIAGNOSIS OF PULMONARY INFECTIONS

Pulmonary cytologic samples are of diagnostic value in a variety of lung infections.[2,37,38] Pathogenic and opportunistic microorganisms may be seen in routine Papanicolaou-stained preparations. Special stains are easily applicable to smears and cell blocks, affording a more precise characterization of the agent.

Pathogenic Microorganisms

Bacteria are difficult to recognize in Papanicolaou-stained cytologic preparations, and the physician should not render a definitive diagnosis of bacterial infection. However, unstained or decolorized smears may be stained with Brown and Brenn, acid-fast stain (*i.e.*, Kinyoun), or with acridine orange for examination under fluorescent light. The latter procedure permits excellent visualization of free and intracellular rods and cocci. Nevertheless, differentiating in sputum specimens between a significant pathogen and the normal flora from the oral cavity remains difficult at best.

Viral infections are more easily diagnosed in cytologic preparations because of associated nonspecific changes and pathognomonic virus-induced cellular alterations.[37,39,40] Regenerative changes affecting bronchiolar epithelial cells are nonspecific but raise the suspicion of a viral process. Ciliocytophtoria is another nonspecific change that has been linked to viral infection.[9] The finding of characteristic intranuclear and cytoplasmic inclusions is diagnostic of herpes simplex, cytomegalovirus, or adenovirus (see Chap. 42).

Fungal infections diagnosable in pulmonary cytologic specimens include cryptococcosis, blastomycosis, coccidioidomycosis, and histoplasmosis.[37,41,42] The size, staining, and occurrence of the organisms in relation to macrophages help to establish the diagnosis (see Chap. 43).

Opportunistic Infections

Immunosuppressed hosts may suffer from infections by saprophytic fungi such as *Candida, Aspergillus, Zygomycetes, Nocardia*, and *Sporothrix schenkii*.[2,43] Organisms belonging to the genus *Candida* appear as regularly constricted pseudohyphae, often accompanied by numerous yeast forms (*i.e.*, blastoconidia). Less commonly, recognizable germ tubes are identified in sputum specimens. *Aspergillus* species are recognized by the presence of hyphae with true septation, which have a uniform caliber and characteristically show Y-shaped, 45-degree branching. Rarely, the hyphae of *Aspergillus* species produce a large quantity of oxalate crystals, which are entrapped among the hyphae. Mucormycosis is diagnosed by the identification of irregularly branching hyphae of *Zygomycetes* species, whose variable diameter resembles smashed and twisted cellophane ribbons. *Nocardia asteroides* is seldom seen in sputum specimens, but bronchial washings may dislodge sulfur granule-like structures which contain matted, filamentous pseudomycelia.

The patient with acquired immunodeficiency syndrome (AIDS) is prone to infections with *Mycobacterium avium-intracellulare*, which is diagnosable by BAL. The negative image of *M. avium-intracellulare* microorganisms within macrophages is characteristic (Color Fig. 3-3). However, patients taking the drug clofazimine may also present with similar inclusions that should not be confused with *M. avium-intracellulare*.[44,45] *Pneumocystis carinii* is a notorious protozoan commonly involved in pneumonias of the AIDS patient and frequently seen in cytologic preparations, particularly BAL.[33,42,46]

Less common infection of immunosuppressed patients is caused by the nematode *Strongyloides stercoralis*.[47] Parasitic pulmonary diseases such as echinococcosis, paragonimiasis, ascaridiasis, and teniasis can also be diagnosed cytologically (see Chap. 44).

FINE NEEDLE ASPIRATION

FNA is indicated primarily in the diagnosis of tumors. For benign localized lesions, such as granulomas, FNA is an alternative to thoracotomy. The yield in diffuse infiltrative processes is much

smaller, but the procedure is valuable in evaluating the immunosuppressed host who is too ill for open biopsy.

Technique

It is crucial to provide assistance to the radiologist on an on-call basis; a minimum warning time of 1 hour before the FNA is necessary. A mobile cytopathology unit consisting of staining dishes, fixative bottles, glass slides, and a microscope should be placed in a cart located in the radiology department. The pathologist consults with the radiologist before the sampling of the lesion and must be aware of the needle used. A 22- to 24-caliber needle is most appropriate, but some physicians prefer the Rotex needle, which obtains larger fragments of tissue by a rotating motion. A small amount of the material should be examined to ascertain its adequacy. It is important to observe the drop of material on the slide before making the smears. If the material is thick, a slide is placed horizontally over it, and the two slides are pulled apart. If the material is thin, the edge of a glass slide is applied against the drop and pulled over, in the manner employed to prepare a peripheral blood smear. This tends to leave the fluid portion behind and concentrates the cells on the opposite end of the slide. A drop of the material is expelled onto a slide and spread and stained by the fast Papanicolaou procedure. Other rapid staining methods that yield good results include Diff-Quick and toluidine blue.

After good cellularity has been ascertained by microscopic examination, additional smears are made and placed in 95% alcohol. The needle contents are washed thoroughly in Plasmalyte, which is the medium for transportation. In the laboratory, the Plasmalyte is divided in three parts: one portion is placed in 95% alcohol and processed by Gelman filtration or cytocentrifugation for light microscopy; one portion is placed in glutaraldehyde and processed by microcentrifugation for electron microscopy; and one portion is placed in B-5 fixative for cell blocks to be stained by cytochemical and immunocytochemical methods.

For electron microscopy, the material is centrifugated in a Microfuge, the pellet is embedded in plastic, and sections are cut and stained with toluidine blue. Cutting various levels of the block and examining each of them markedly increases the chances of obtaining malignant cells.

Interpretation

The first rule is that criteria learned with other cytologic specimens do not necessarily apply to this method. The background plays a major role in the interpretation, as does the cellularity of the specimen. A necrotic background without many inflammatory cells but composed mostly of watery or amorphous proteinaceous debris indicates neoplasia. A heavy, mixed inflammatory infiltrate favors infection. Granulomas result in frequent dry passes and poor cellular samples, but malignant tumors often yield copious material.

Another major consideration is the type and homogeneity of the cell population in the FNA. Malignant tumors are more likely to appear as a single-cell population of a type alien to the aspirated area. The tumors should be classified according to criteria similar to those applied to surgical specimens. We follow a modified WHO classification based on the eclectic use of cytomorphologic and ultrastructural features.

Diagnosis of Tumors

SQUAMOUS CELL CARCINOMA

Tumor cells of poorly or nonkeratinizing squamous carcinoma tend to spread in sheets in FNA, but keratinized malignant cells exfoliate individually, because the tumor cells lose cohesiveness as they undergo centripetal maturation and degeneration. In the sheets, intercellular bridges may be observed, and the cytoplasm appears cyanophilic or orangeophilic, depending on the degree of differentiation. Cellular shapes vary, but isolated, elongated elements with caudate and bizarre cells predominate (Color Fig. 3-4). The nuclei follow the general shape of the cell and display sharp borders and marked hyperchromasia. Nucleoli are only slightly prominent, and the chromatin appears coarse in well-differentiated neoplasms. Occasionally, mature noncohesive cells derived from a necrotic tumor may reveal degeneration, but a large number of resistant tonofilaments survive and permit recognition of the tumor as squamous cell carcinoma (see Chap. 48).

ADENOCARCINOMA

In adenocarcinoma, the cells appear single or in cell balls with marked predominance of the latter. Acini, minibiopsies, or sheets may be observed; the needle aspirates tend to be highly cellular in this type of neoplasm as a result of the friability of the tumor. However adenocarcinoma does not have the fragility of small cell carcinoma nor the loss of cohesiveness of squamous cell neoplasms. The cytoplasm is often foamy or vacuolated and invariably cyanophilic, but some tumors unexplainably acquire a maroon color, which may cause confusion with squamous cell tumors. The pitfall of making a diagnosis of mixed or adenosquamous carcinoma for such small cellular samples should be avoided. Unlike squamous cell carcinomas, in which the major axis of the cells is parallel to each other within the sheets, adenocarcinoma cells are arranged in various directions and tightly wrapped around one another. This produces scalloped community borders, a large amount of cytoplasm that appears to be shared by more than one cell, and nuclear molding caused by the impressions nuclei make on each other. The groups often have overlapped nuclei, a phenomenon referred to as tridimensionality (Fig. 3-9). Nuclear borders are delicate, but chromatin deposits may result in focal thickening. The chromatin pattern is fine, and there is attenuate clearing and clumping of the chromatin granules. Nucleoli are large, eosinophilic, and frequently spiculated (see Chap. 47).

BRONCHIOLOALVEOLAR CARCINOMA

Bronchioloalveolar carcinomas tend to display cell clusters in FNA that are indistinguishable from those of centrally arising adenocarcinoma. The cytoplasmic texture is fluffy and may actually reflect intracellular luminal spaces (Fig. 3-10). Occasionally, the tumor forms loose sheets of cells with abundant cytoplasm and nuclei showing inclusions (Fig. 3-11). Using electron microscopy, bronchoalveolar carcinomas may be subtyped according to their secretory granules.[48] In the type II cell variant, the granules show concentric lamination identical to that of the lamellar bodies of type II pneumocytes. These tumors stain positively with an antibody against surfactant apoprotein by the peroxidase-antiperoxidase technique. In Clara cell tumors, the granules are electron dense and lack a double limiting membrane. They are commonly

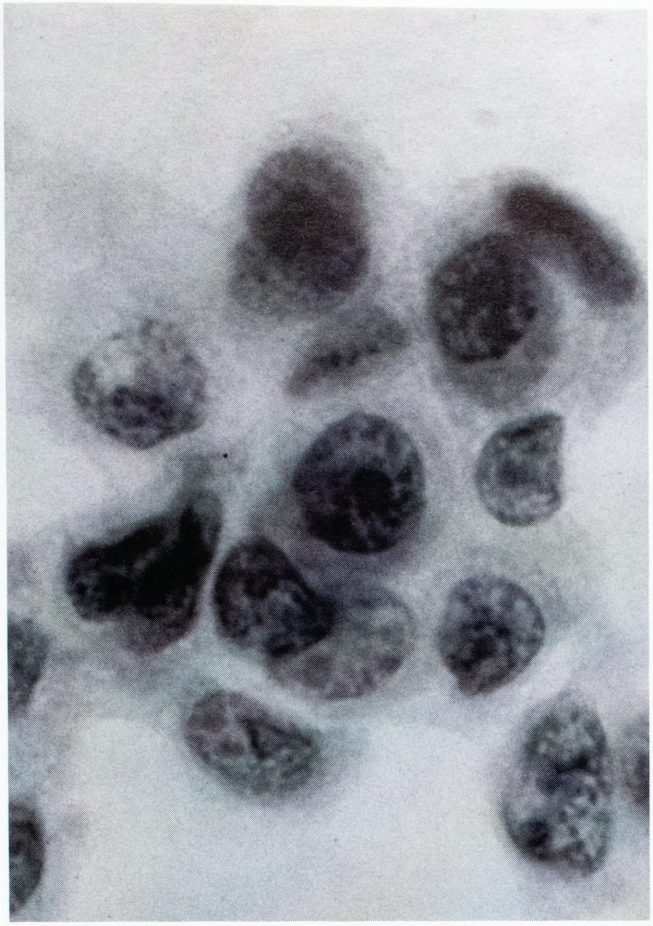

FIGURE 3-9. Adenocarcinoma cells found in a bronchial washing specimen show nuclear molding, and prominent macronucleoli. (Papanicolaou stain; oil immersion view; original magnification ×1000.)

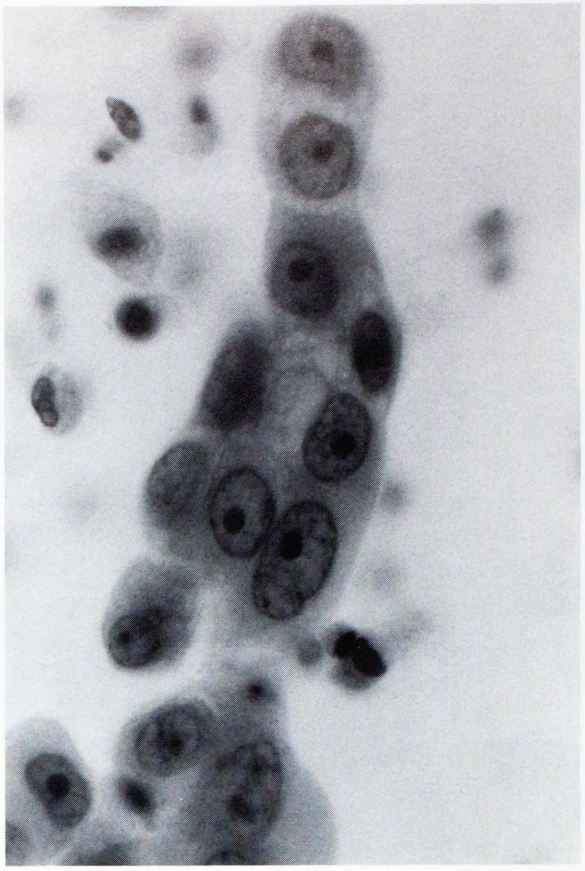

FIGURE 3-10. Papillary formation occurs in bronchioloalveolar carcinoma. The clear areas in the cytoplasm are intracellular lumina. (Papanicolaou stain; high magnification.)

found in the vicinity of clear spaces thought to have contained glycogen and may display a fingerprint pattern (see Chap. 47).

SMALL CELL CARCINOMA

The cells in small cell carcinoma appear be isolated or in small, loosely formed clusters more appropriately called pools. The cells are molded in small groups of a few cells each, but there are spaces among them so that the aggregates vary considerably in size and are never as tight as those of adenocarcinomas (Fig. 3-12). The two axes of the cells are almost equal in length, and their orientation is difficult to appreciate. The cytoplasmic rim is practically invisible leading to nuclear-cytoplasmic ratios that are the largest of any epithelial neoplasm, and there are no intercellular bridges.

In the intermediate variant of small cell carcinoma, wisps of frayed cytoplasmic material less cyanophilic than the nucleus are identified. The most salient cytologic feature is the absence of a nucleolus in more than 80% of the cells. If present, nucleoli are inconspicuous and small, except in intermediate tumors, in which they are slightly larger and more readily discernible in a greater proportion of the cells. Because of the fragility of the cells, these samples often show strands of cyanophilic amorphous material, which are the cytologic counterpart of a crushing artifact.[49] Under oil immersion, the nuclei appear more irregular than initially perceived, and chromatin granules are perceptible with little clearing among them and frequent chromocenters.

In our practice, the diagnosis of poorly differentiated carcinomas (*e.g.*, non–small cell type) is rendered if small cell carcinoma is excluded by light microscopy but it is not possible to identify a subtype. In these cases, electron microscopy is performed within 24 hours to further classify the tumor as poorly differentiated squamous or poorly differentiated adenocarcinoma.

LARGE CELL CARCINOMA

Large cell undifferentiated carcinoma is composed of very large cells that shed individually, but they may occasionally form enormous flat sheets. These groups lack the three-dimensional appearance of adenocarcinomas and have minimal nuclear overlap. The cytoplasm is less dense than in squamous cell carcinoma; it is frequently foamy but seldom as vacuolated as in adenocarcinoma. There is a lack of tinctorial definition, and cyanophilia or slight orangeophilia is observed. The nuclear-cytoplasmic ratio is high, but the large size of the cells and the presence of macronucleoli permit differentiation from small cell carcinomas (Fig. 3-13).

The chromatin distribution is extremely variable, with clear

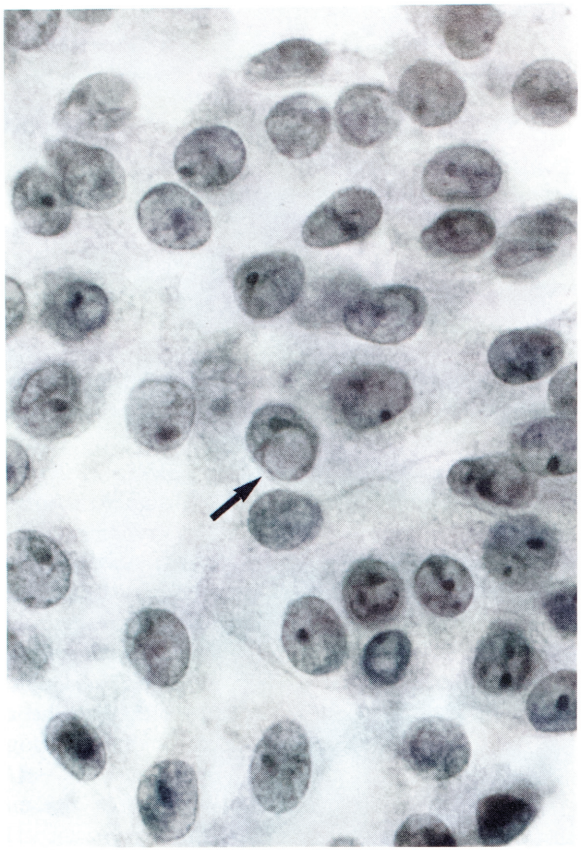

FIGURE 3-11. Bronchioloalveolar carcinoma discovered in a fine needle aspiration sample shows abundant cytoplasm, bland nuclei, and pseudoinclusion (*arrow*). (Papanicolaou stain; high magnification.)

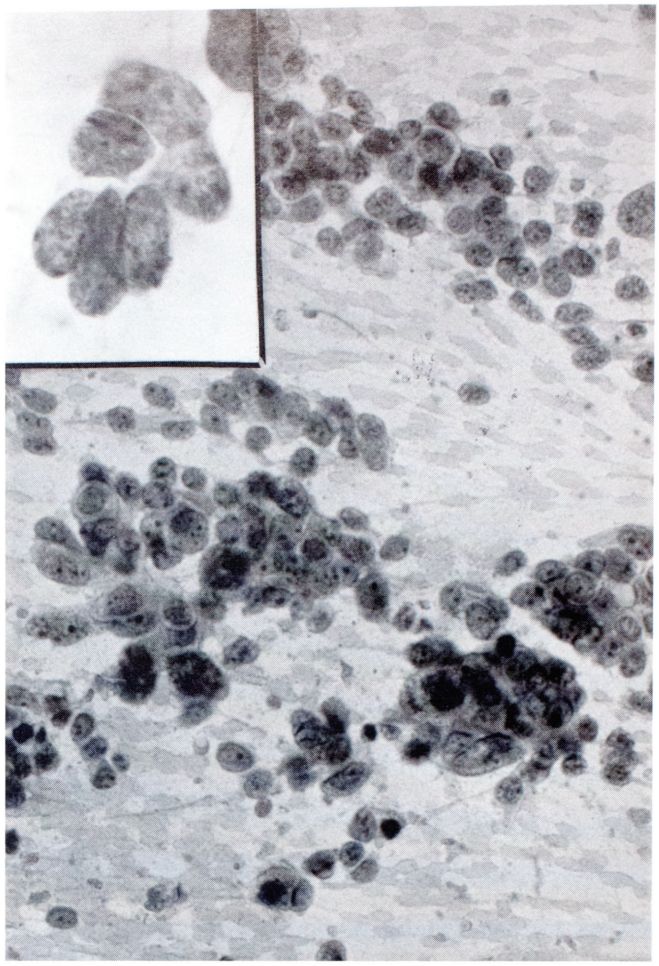

FIGURE 3-12. This loose group of cells is from a small cell carcinoma. Nucleoli are lacking in nuclei that are slightly molded to one another (*inset*). (Papanicolaou stain; oil immersion view; original magnification $\times 1000$.)

and clumped patterns seen within the same tumor. The greatest help in identifying this tumor is gauging its enormous size against built-in yardsticks such as lymphocytes or erythrocytes. Tumors revealing a high proportion of large, multinucleated giant cells have been called giant cell carcinoma. Frequently, large cell undifferentiated and giant cell carcinomas of the lung are variants of poorly differentiated adenocarcinoma as shown by their focal mucin positivity and frequent ultrastructural finding of microvilli on the cell surface (see Chap. 50).

NONEPITHELIAL TUMORS

Solitary or multiple pulmonary lesions are not necessarily malignant, and even malignant lesions may not represent carcinoma. Granulomas and hamartomas should be ruled out, and they are commonly the target of FNA. Hamartoma of the lung may contain a variety of the tissue, including fibroblastic cells engulfed in an acellular myxoid matrix.[50] Most pulmonary hamartomas are cartilaginous, and mature chondrocytes may be represented in FNA (Fig. 3-14). In practice, only lipomas and neurilemomas can be diagnosed cytologically, but they are exceedingly rare. Primary sarcomas of the lung, including chondrosarcoma, malignant fibrosis histiocytoma, carcinosarcoma, pulmonary blastoma, and Kaposi sarcoma have been well documented in the lung, but they are not frequently seen in aspirated material (see Chap. 56).[51–53]

METASTATIC NEOPLASMS

The lung is the most frequent site of distant metastasis from many primary sites.[54,55] Metastatic lesions of the lung are more frequent than primary tumors if common sources such as the breast, gastrointestinal tract, pancreas, kidneys, and various muscle sites are taken into account. Melanomas and germ cell tumors often metastasize to the lung. Lymphomas metastatic to the lung tend to be high-grade tumors (see Chap. 60).

Metastatic carcinomas may be difficult to differentiate from primary lung carcinomas. Knowledge of the patient's previous history considerably facilitates this diagnosis, and a combination of electron microscopy and immunocytochemistry may assist the determination. Renal cell carcinomas may be difficult to differentiate from large cell anaplastic carcinoma of the lung and mesothelioma (Fig. 3-15; see Chap. 57).

Metastases of sarcomas are characterized by bizarre, large, noncohesive cells that may not allow classification of the neoplasm (Fig. 3-16). Comparison with preexisting material is critical in establishing the nature of these tumors.

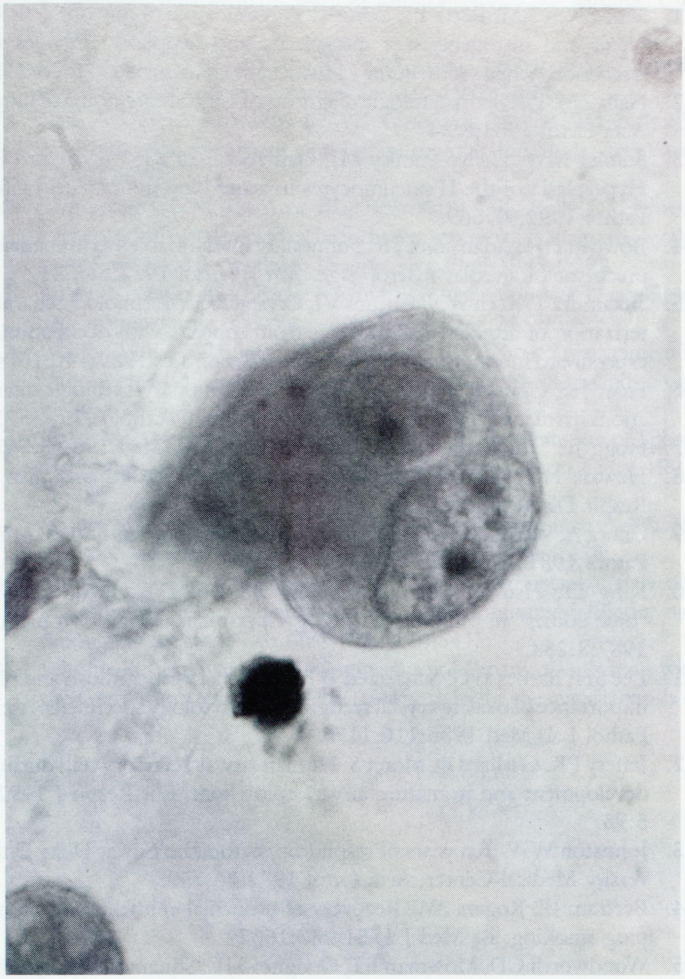

FIGURE 3-13. Large cell carcinoma is indicated by an enormous binucleated cell with prominent nucleoli. (Papanicolaou stain; oil immersion view; original magnification × 1000.)

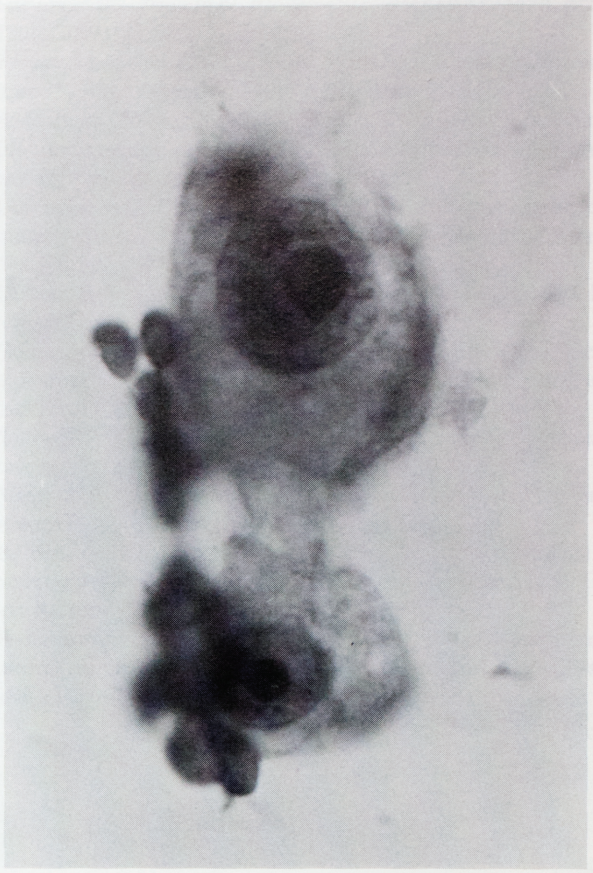

FIGURE 3-15. Metastatic renal cell carcinoma is indicated by these two large cells with foamy cytoplasm and striking macronucleoli. (Papanicolaou stain; oil immersion view; original magnification × 1000.)

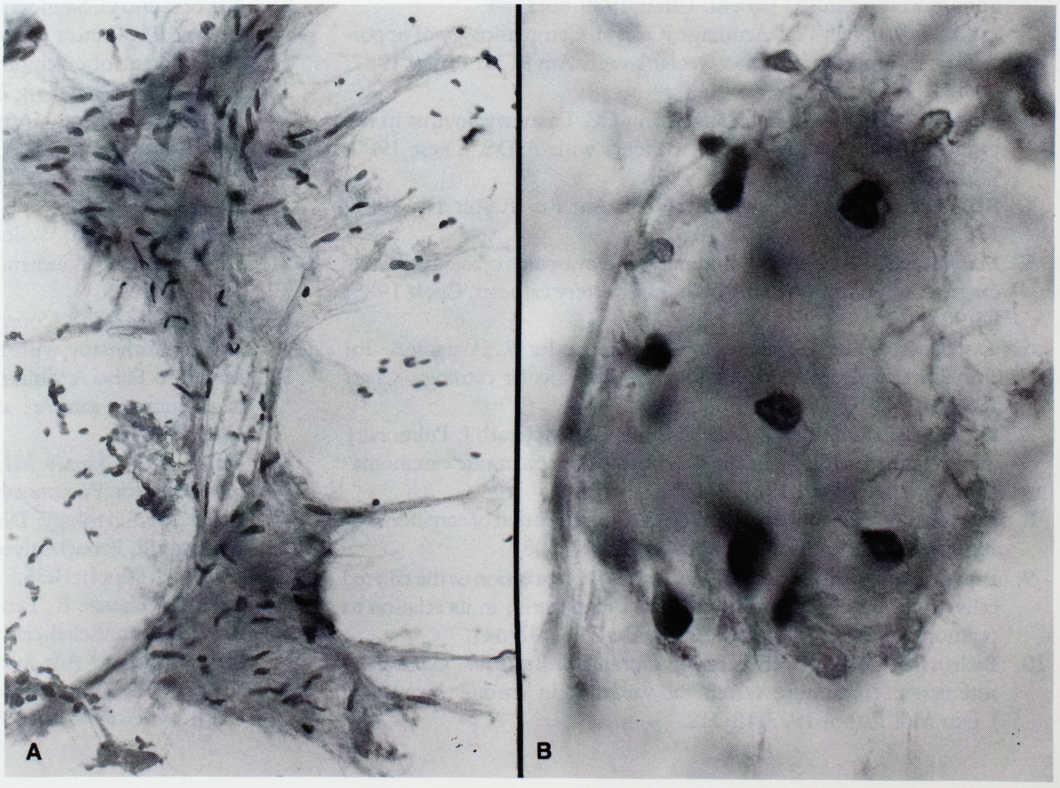

FIGURE 3-14. Fine needle aspiration of chondromatous hamartoma. (**A**) Spindle component with myxomatous stroma. (Papanicolaou stain; low magnification.) (**B**) Chondrocytes in a cartilaginous matrix. (Papanicolaou stain; high magnification.)

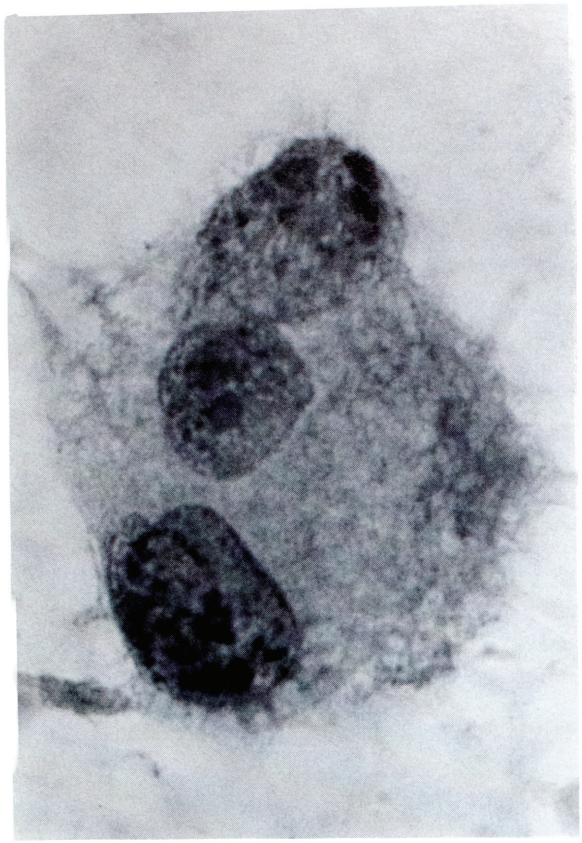

FIGURE 3-16. Malignant fibrous histiocytoma is composed of large, multinucleated malignant cells with abundant, finely textured cytoplasm. (Papanicolaou stain; oil immersion view; original magnification × 1000.)

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