REVIEW ARTICLE

THE CYTOPATHOLOGY OF THE RESPIRATORY TRACT

The Cytopathology of the Respiratory Tract

A Review

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DURING THE FIRST FIVE YEARS of this decade, the cytopathology laboratories of Duke University and The Medical College of Virginia examined over 30,000 specimens from the lower respiratory tract, a number exceeded only by that recorded for examination of smears from the female genital tract. These statistics are a true reflection of the frequency with which cytologic diagnostic techniques of the lower respiratory tract are being utilized in hospitals and clinics throughout the country. The major purpose of this review article is to discuss the *raison d'être* of this evergrowing specialty. After a brief description of historical background, the current status of the following areas will be discussed: preparatory techniques, cellular changes associated with nonneoplastic disease, the cytopathology of primary and metastatic cancer, screening and diagnostic accuracy, and new horizons. Particular attention will be given to those developments which have occurred during the past 10 years.

Two earlier reviews (Grunze, 1960 and Russell *et al.*, 1963) thoroughly summarize cytologic developments in pulmonary cytology up until their respective times.[1,2]

Historical Background

It is an interesting footnote to medical history that the year 1838 witnessed not only Schleiden's publication of his cell theory[3,4] but also the publication of two of the earliest studies on the microscopic examination of exfoliated cells. The first was by Donné on fresh smears prepared from human colostrum.[5] The second was a book by Mueller in which considerable discussion was devoted to the microscopy of cancer cells.[1,6] With these studies there began a flurry of investigative activity in microscopy of body fluids and exudates, with secretions from the tracheobronchial tree in particular arousing much interest.

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In 1845 Donné published the first work dealing with exfoliated cells of the respiratory tract.[7] Walshe in London in 1846 made note of the presence of tissue fragments of malignant tumor in sputum.[8-10] Beale in 1860 demonstrated malignant cells in the sputum from a patient with cancer of the pharynx.[10-12] The work of Hampeln did much to further strengthen the validity of cytologic diagnosis. In 1887 he published the report of a case in which cancer cells were correctly recognized in the sputum 5 months before the patient's death. The origin of these cells in a bronchogenic cancer was confirmed at autopsy. In 1919, Hampeln was the first to publish a series of cases in which sputum cytology was utilized for the primary diagnosis of lung cancer. In these series, cytology was positive for tumor cells in 13 of 25 cases of lung cancer.[1,2,8,10] Thus, as the end of the nineteenth century approached, significant work already had been done which should have firmly established diagnostic cytology as a useful and important tool in modern medicine. Unfortunately, however, that was not to be the case. The utilization of this methodology went into great decline in the early part of the twentieth century, and in spite of sporadic publications, was not to be revived until the landmark publication of Papanicolaou and Traut in 1943.[13] Two major causes for this decline have been cited: The development of cultural methods for microorganisms and the invention of the mechanical microtome diluted the interest in direct microscopic examination of smears.[14] Also, some late nineteenth century medical thought questioned the morphologic identity of cancer cells as contrasted with benign ones.

Although contributions to the exfoliative cytologic literature were sparse during the first 40 years of the twentieth century, two papers merit attention here, as they jointly contributed to a more solid technical foundation. In 1928 Papanicolaou reported on a simple method of fixing vaginal smears with which fine cytologic detail could be permanently retained.[15] Similarly, Dudgeon and Wrigley in 1935 successfully applied a wet-film fixation in a mixture of ethyl alcohol, mercuric chloride, and acetic acid to the examination of sputum for neoplastic cells.[16]

It is now generally accepted that the Papanicolaou and Traut monograph of 1943 was the catalyst which provoked a rediscovery of exfoliative cytology, a tool which had lain in disuse for 50 years. As has been cited by Foot, the late 1940s and early 1950s marked a period of development for pulmonary cytology.[17] Numerous papers were written to report on the results of new techniques and the ability to detect neoplastic cells and predict histologic type of neoplasm. Of particular significance in these areas are the contributions of Wandall,[8] Banforth,[18,19] Woolner and McDonald,[20-27] Papanicolaou,[28-30] Farber and his associates,[10,31-35] Clerf

and Herbutt,[36-42] Foot,[17,43] Umiker,[44-48] Richardson and his associates,[49,50] and Koss.[51,52]

Techniques

As is true for most of the biomedical sciences which utilize morphology, excellent cytopreparatory techniques are mandatory for interpretive accuracy. A specimen from the respiratory tract which has been prepared for cytologic diagnosis should a) exhibit an abundance of well-preserved and stained diagnostic cellular material, b) have been prepared rapidly and with relative ease, and c) remain preserved for permanent slide storage. Many laboratories have studied techniques for the best realization of these criteria. Paraffin embedding and sectioning of sputum [53-55] is in the experience of the authors the worst possible technical approach to respiratory cytology. Various techniques for freeing and concentrating tumor cells by mucolysis were commendable in concept but were frequently too laborious and time consuming to be practical.[56-69] Three major techniques have stood the test of time and are the most widely utilized today: the wet-film preparation and fixation from fresh or prefixed respiratory material, the Saccomanno method,[70,71] and membrane filtration.[72-77] These methods are used for spontaneously produced sputum, induced sputum,[78-94] and bronchial washings and brushings. The Papanicolaou method is the most generally accepted staining method.[95] In the United States, in particular, it has gained virtually universal popularity.

For the first technique cited, a fresh, early morning, "deep cough" specimen of sputum is collected into a shallow, wide-mouthed sputum jar and brought immediately to the laboratory. It is examined grossly for tissue fragments and other suspicious areas. Smears from these areas and others randomly sampled are prepared by gentle, even spreading of the specimen between two glass slides until a thin uniform smear is obtained. These are fixed immediately, without air drying, in 95% ethyl alcohol for 15 to 30 minutes. At the operating table, direct smears are prepared from the bronchoscope and from the brush. The unfixed fresh bronchial washing is brought to the laboratory for membrane filter preparation. The brush is agitated in a balanced salt solution, and from this solution membrane filters are prepared.

In those situations in which it is not possible to transmit unfixed material to the laboratory, prefixed sputum may be obtained by instructing the patient to expectorate into a sputum jar half filled with 70% ethyl alcohol.

The Saccomanno method also involves the collection of induced or spontaneously produced sputum with some prefixation. This is done using

a wide mouth jar containing 50% ethyl alcohol with 2% polyethylene glycol (Carbowax). The patient coughs directly into this bottle, insuring some immediate fixation of the cellular material. Collections may be single specimens or they may be collected over several hours. Once the specimen reaches the cytology laboratory the major preparatory steps are blending in a Waring-type blender for several seconds and centrifugation of the resulting specimen. Blending breaks up the mucus that may be present, while some concentration of cellular material occurs with centrifugation. As smears are made from the concentrated cellular material, a large number may be prepared from a single specimen. This is an advantage in screening projects where abnormal cells may be present in limited numbers and in the preparation of teaching slides from interesting cases. This method has been used at the Medical College of Virginia almost since its publication in 1963 but until recently has been used by few other laboratories. The technique has allowed Saccomanno and his colleagues to collect a large repository of case material documenting the development of lung cancer in certain high-risk groups. Recently launched projects to screen high-risk groups for lung cancer are using the Saccomanno collection technique as their primary methodology.[96,97] Criticism has been leveled at the method because of potential destruction of cells during blending and the possibility of laboratory infection in dealing with contaminated material, particularly from patients with tuberculosis. The authors' experience with the method in comparison with fresh sputum samples in the same patients has shown a richer harvest of neoplastic cells with the blending technic in the majority of cases. Specimen seeding experiments by Saccomanno followed by blending and cell counts indicate negligible cell loss. Cells are not fragmented or destroyed by the blades of the blender, but convection currents are set-up which produce a homogeneous mixture of cell material and debris that is much easier to distribute evenly on slides.

Where clinical findings support a primary diagnosis of respiratory infection, particularly a fungus infection, fresh sputum for cytologic examination has some advantages. The blending technic tends to disperse and destroy fungal organisms, primarily those with a mycelial tissue phase such as aspergillus. The staining quality of primary pathogenic organisms like *Blastomyces dermatitidis* may be less crisp, making detection of the organism on any given slide more difficult. With the growing popularity of bronchial brushing, more cytology laboratories are receiving both fresh and prefixed material, and the few objections to the Saccomanno technic are overcome.

Documentation of an increased risk of infection of the cytology labora-

tory personnel using the blending technic is lacking. At the Medical College of Virginia there has been 1 case of tuberculosis in 12 years in a staff of 10 cytotechnologists. Clinically, that case strongly suggests reactivation of prior tuberculosis. With this laboratory processing an average of 2000 respiratory specimens per year, and having an active tuberculosis unit until very recently, that represents an exposure of 2400 specimens per technologist over the 12-year period. Obviously, it is wise to observe common sense precautions in dealing with potentially infectious material no matter what cytologic preparatory method for respiratory specimens is favored.

Most important to the cytotechnologist and cytopathologist is the difference in cytologic presentations that may occur with fresh versus Saccomanno-prepared specimens. With certain types of lung cancer and with reactive lesions that may be misdiagnosed as lung cancer, differences in preparation of the cytologic specimen may effect the cellular detail, cellular characteristics, and spatial arrangements of cells. Recognizing these differences and taking advantage of them will lead to more correct specific diagnosis of lung cancers and avoidance of the misdiagnosis of reactive proliferative processes within the lung as lung cancer.

As an adjunct to the fresh sputum and Saccomanno techniques, membrane filtration is useful for the harvest of cells from material obtained by instrumentation and from thin watery sputum.

Cytopathology of Benign Respiratory Diseases

As the production of sputum in quantities sufficient to be expectorated does not occur in the absence of respiratory disease, the examination of such specimens generally demands a greater challenge than that encountered in the screening of genital smears from asymtomatic women. Although sputum production in itself is an abnormal phenomenon, its cellular components may be unremarkable. The basic morphology of these cellular components has been well described in the literature by Farber and his associates,[34] Woolner and McDonald,[20-27] Koss,[99] and more recently by Frost and his associates.[100] The normal epithelial components of sputum consist of squamous epithelial cells exfoliating from the oral cavity and pharynx, and respiratory columnar epithelium exfoliating most frequently from the tracheobronchial tree and less frequently from the upper respiratory passages, terminal bronchiolar epithelium, and alveolar cells. The tall columnar cells lining the tracheobronchial tree may be of two varieties. Most frequently encountered are ciliated columnar cells. They are most commonly seen in bronchial washings, aspirations, or brushings. They should not be present in large numbers in sputum except

in postbronchoscopy sputum specimens. They are characterized in profile by a columnar or prismatic shape ending in a tail. The nucleus is oriented toward the tail end and shows a finely granular chromatin pattern with one or more small nucleoli. Cilia with a terminal plate are present (Figure 1). During degeneration the cilia are often lost, leaving the cell with just a terminal plate. Encountered less commonly are mucus-producing bronchial cells, so-called goblet cells. In profile, a large hypersecretory vacuole which distends the cytoplasm and distorts the nuclear shape can be observed in that portion of the cytoplasm between the nucleus and the luminal portion of the cell. These cells are more common in patients with chronic tracheobronchial disease such as asthmatic bronchitis and bronchiectasis. "Irritation forms" of bronchial epithelium may occur in response to a host of insults varying from microbiologic pathogens to environmental irritants.[100-110] The cells may become markedly enlarged, with a coarsening of the nuclear chromatin pattern and the appearance of one or more enlarged nucleoli. The presence of a terminal plate with cilia, although quite degenerate, may be of aid in assuring the examiner that the cell is benign (Figure 2, left). Another common response to irritation is multinucleation.[111,112] Nuclei are small and mirror image (Figure 2, right). Although such cells may appear after a wide variety of insults, including chemical agents, infection, and ionizing radiation, they are most commonly seen following instrumentation, particularly bronchoscopy. Hyperplasia of these bronchial cells may occur in response to a number of chronic diseases of the lung, particularly bronchiectasis,[113] chronic bronchitis, and asthma.[114,115] It was in patients with chronic asthmatic bronchitis that the papillary tissue fragments exfoliating from such hyperplasia were first noted and mistakenly diagnosed as adenocarcinoma. Indeed, these fragments are said to appear in the sputum from 42% of cases of asthmatic bronchitis. The cytologic presentation is that of papillary clusters of cells partially covered on the surface by well-differentiated ciliated respiratory epithelium. There is some nuclear molding between individual cells although intranuclear chromatin and nucleolar structures remain relatively unremarkable. A varying number of highly vacuolated mucus-producing cells may also be present in these fragments. It is extremely important that they be accurately recognized and not be mistaken for adenocarcinoma. The key to their benignancy is to be found in the finely granular chromatin pattern, regular uniform nucleoli, and the presence of cilia (Figure 3).

The least frequently encountered epithelial components in sputum are cells exfoliating from the terminal bronchioles and from the alveoli. Considerable attention has been given in the literature to the association

of these cells with such pulmonary problems as pulmonary fibrosis, thermal injury, thromboembolism with or without pulmonary infarction, anthracosis, and chronic organizing pneumonia. The relationship of these epithelial changes to the development of bronchioloalveolar cell carcinoma has been the subject of much interest, and the presence of these cells in sputum may represent a particularly difficult problem in interpretation to the cytopathologist.[116-121]

Although the utilization of such relatively modern techniques as transmission and scanning electron microscopy and biochemical techniques have enabled us to differentiate a variety of subtypes of terminal bronchiolar and alveolar cells, conventional light microscopy of cytologic specimens does not permit the observer to appreciate these various cell types. Practically speaking, the terminal bronchiolar and alveolar cells are probably not recognized in sputum when they are present in an unaltered form. These cells are relatively small and when present in sputum appear as rounded single cells with finely vacuolated cytoplasm and centrally placed nuclei without abnormalities. As such they are usually mistaken for alveolar macrophages. Only in the presence of insult do they enlarge and become problematic. In such circumstances, they may be formed together as small papillary tissue fragments, individual cells of which show enlarged centrally placed nuclei with one or more stainable nucleoli. The cytoplasm may be granular or finely vacuolated, or may exhibit one or more hyperdistended vacuoles. Differential diagnosis of such cells then becomes a rather formidable problem of determining whether these cells are coming from some of the above named benign disease processes or whether they are derived from a bronchioloalveolar cell carcinoma. Pulmonary infarcts in particular may give rise to cells of this description which are known, to the experienced cytopathologist, to be one of the most dangerous sources for diagnostic error (Figure 4).

The concept of squamous metaplasia of the respiratory mucosa has been applied to a spectrum of alterations beginning with reserve cell hyperplasia and ending with a stratified and keratinized covering resembling squamous epithelium. Its occurrence in individuals exposed to varying environmental toxic agents, particularly cigarette smokers, and its possible relationship to the pathogenesis of bronchogenic carcinoma have become of increasing interest to investigators.[99,100,102-110,122-131]

In association with alterations of the covering columnar epithelial cells previously noted, there begins a proliferation of reserve cells such that a multilayered epithelium, so-called reserve cell hyperplasia, is produced. This epithelium intervenes between the overlying columnar epithelium and the basement membrane. Exfoliation of the columnar cell layer

eventually occurs, leaving behind an epithelium composed of immature reserve cells. As they in turn gradually mature, an epithelium is produced which more and more resembles a squamous type, with cell flattening, karyopyknosis, and keratin production. Reserve cell hyperplasia in cytologic materials is recognized by the presence of tissue fragments composed of small, uniform, tightly coherent cells possessing darkly stained nuclei and a thin rim of faintly cyanophilic cytoplasm. There is nuclear molding, but uniformity exists throughout the fragment. There is no tendency toward fragmentation of the cluster. Toward the edge one may see some maturation and more columnar configuration (Figure 5). At times they may be alarming in appearance and must be distinguished from small cell anaplastic carcinoma [132] (see Figure 28). Other small cell neoplasms, notably leukemias and lymphomas should not be confused with reserve cell hyperplasia, as they characteristically shed into the bronchopulmonary material as single cells.

Cells derived from squamous metaplasia may be seen as single cells and as small tissue fragments. In the latter the cells are grouped in a uniform, monolayered cobblestone-like arrangement with striking uniformity between the cells. Although they resemble mature squamous cells, they are smaller and possess a higher nuclear/cytoplasmic ratio. As squamous metaplasia mimics maturing squamous epithelium, metaplastic cells of varying degrees of maturity may be present. The cytoplasmic staining characteristics may vary from a deep cyanophilia to an organgeophilia, indicating maturation and keratinization of the cytoplasm. The nuclei may be intensely karyopyknotic (Figure 6).

The pulmonary alveolar macrophage and its biologic significance to the cytopathologist have recently been summarized by Frost and his associates.[100] Like alterations of the bronchial mucosa, abnormalities of these cells are becoming of increasing interest in studies of noxious environmental inhalants.[100,133-135] The presence of these cells is mandatory in establishing the satisfactory nature of a sputum specimen. These macrophages are recognized by the eccentric position of the nucleus, which is barely touching the cytoplasmic membrane. There may be abundant foamy cytoplasm and phagocytized material, usually carbon. On occasion the nuclei may assume a bean shape and show one or more nucleoli. Other cytoplasmic inclusions have been noted in the presence of environmental pollutants. Binucleate and multinucleate giant macrophages are not infrequently encountered (Figure 7). These latter cells may be seen in association with chronic lung disease of many varieties, including sarcoidosis, tuberculosis, and other granulomatous diseases, but they are not indicative of any of these and may be seen in the sputum in the absence of

clinical disease. Large vacuoles containing fat have been reported in these macrophages in the presence of lipoid pneumonia.[136,137] Other cells originating from circulating blood which may be seen in bronchopulmonary material include lymphocytes of varying degrees of maturity.[138] They may be associated with a chronic inflammatory process or with the rupture of a lymphoid follicle in the wall of the bronchus. The lymphocytes often stream out in the mucous strands, mimicking the exfoliation pattern of small cell anaplastic carcinoma. The lymphocytes may be distinguished from the small cell undifferentiated neoplasm by failing to mold to one another, showing no intercellular recognition, and lacking malignant criteria. Often when a follicle is ruptured, one will also find phagocytic reticulum cells and capillaries present, and a diagnosis of chronic follicular bronchitis may be made.

Infectious Disease

The awareness that cytologic technics can play a significant role in the primary diagnosis of infectious diseases has been steadily increasing.[139-145] The diseases in which cytology has been of greatest usefulness are those in which the responsible microorganism has a morphology which is specific enough to be detected by light microscopic methods or produces specific cellular changes. Cytopathology generally has had very little to offer to the study of bacterial infection. A number of studies have devoted themselves to the exfoliations associated with pulmonary tuberculosis.[145-150] Indeed, on occasion in patients with tuberculosis, one may observe epithelioid cells and Langhan giant cells. But these findings are rarely helpful in establishing the diagnosis. Likewise, searches for acid-fast bacilli in cytologic material from these patients have proven disappointing. On one previous occasion in the Duke Hospital Cytopathology Laboratory, we were able to restain a sputum smear for acid-fast bacilli and detect in several macrophages the presence of acid-fast organisms. These occurred in a child with *Mycobacterium intracellulare* (Battey bacillus). Attempts by us to apply this observation to other patients with atypical mycobacterial disease have been unsuccessful.

Mycoses

With mycotic infections, the role of cytology has become increasingly valuable. In these diseases, the etiologic agent is visible and in many cases has a morphology on which a specific diagnosis may be based. The detection of these organisms in a stained cytologic specimen may be the first clue to the nature of the patient's problems. The accuracy of observation is dependent upon the ability of the cytotechnologist and the patholo-

gist to appreciate the various forms that these organisms may assume. Specific morphology and refractility of cell walls have been the most important criteria for detection. Staining characteristics are too variable to be of help. Although special stains for fungi are useful, with several exceptions they are not necessary. Indeed, it has been established that these organisms are easily seen on a Papanicolaou stain. Mucicarmine or alcian blue helps to visualize the capsule of cryptococcus, but the organism can be identified on the basis of other features. Plane of focus may be another critical factor on which detection depends. Some of the fungi are so thick that they may be blurred or even absent in the plane of focus at which human cells are being studied on the smear or membrane filter.

An organism which has been frequently encountered in our laboratories is *Blastomyces dermatitidis*. In cytopathologic materials which have been fixed in 95% alcohol and stained by the Papanicolaou technique, *B. dermatitidis* appears as single or budding spherical cells 8 to 15 μ in diameter with thick refractile walls. The thickness of these walls, along with some tendency for the cell mass to retract away from these walls, may impart to these forms a "double contoured" appearance. No hyphae are seen. The most important criterion for morphologic confirmation of blastomyces is the nature of the budding. Single budding is characteristic. The bud has a tendency to remain in close apposition to the mother cell so that a flattening of the two apposed surfaces occurs. Staining is highly variable and of little help as an aid in differential diagnosis. The wall is highly refractile and may stain a pale blue-green. The cytoplasm stains variably. In some cells, scattered granules of varying staining qualities may be seen embedded in an otherwise nonstaining cytoplasmic mass (Figure 8). Ultrastructural examination has revealed that these small masses are multiple nuclei. In other cells the entire cytoplasmic mass may shrink within the cell wall and have basophilic staining properties. This shrinkage produces a halo between the cytoplasmic mass and the cell wall which is a useful characteristic as an aid in identification. The unwary may well mistake these yeast cells for those of human origin, the cell wall being mistaken for cytoplasm and the cytoplasmic mass for a nucleus.[151]

Although the authors have most frequently observed the budding yeast forms of *Cryptococcus neoformans* in sputum and in bronchial material, we have also seen it in specimens of urine and cerebrospinal fluid as well. Like blastomyces, single budding is characteristic of this yeast; but in contrast to that of blastomyces, the cryptococcus single bud pinches off, leaving a markedly attenuated isthmus of attachment to the mother cell and thus assuming a tear drop shape. Each cell is ovoid to spherical, with a thickened wall, and measures 5 to 20 μ in diameter (Figure 9). It is often

surrounded by a gelatinous capsule which usually requires a special stain for visualization. However, on occasion, these capsules may stain with the Papanicolaou technique. Not infrequently this organism may be seen in sputum, as well as elsewhere, with virtual absence of inflammatory exudate.[141-145,152]

The spherules of *Coccidioides immitis* may be encountered quite frequently in the sputum of infected patients and are particularly common in sputum from patients in those parts of the country in which the disease is endemic. Spherules and endospores of coccidioides have been reported also in cytologic preparations of gastric washings and cerebrospinal fluid as well as sputum and bronchial washings. The spherules of *Coccidioides immitis* may present a particularly dramatic appearance because of their capability of assuming sizes in excess of 100 μ in diameter. The spherule may be empty or contain endospores. The latter are round, nonbudding structures measuring 2 to 5 μ in diameter (Figure 10). The empty spherules may be confused with nonbudding forms of *Blastomyces dermatitidis*. Arthrospores may be encountered and must be differentiated from those of geotrichum species.[153]

In contrast to some of the other organisms being discussed, *Histoplasma capsulatum* is more easily viewed in cytologic preparations utilizing the aid of special stains. In our laboratories, we have found methenamine silver most useful for this purpose. The organism appears as a 1 to 5 μ round to oval body with budding. For diagnostic purposes it must be engulfed within macrophages or neutrophils.[143,145]

The organisms which have just been discussed are primary pathogens and as such do not appear in pulmonary material in the absence of infection. Such a situation is not necessarily the case for the opportunistic fungi which may also be observed in these materials. These latter groups are usually considered saprophytes or contaminants, but they may invade and produce infection in persons whose resistance has been lowered in some manner. The incidence of this has been increasing in recent years.[154] It is important that their presence be noted so that appropriate further studies may be properly initiated to determine significant infection. Differential diagnosis of these organisms becomes more difficult than for the yeast, as most of the former are characterized by branching or nonbranching hyphal fragments with or without spores.

Among the opportunists, we have observed aspergillus species most frequently. The most characteristic presentation is that of thick, septate hyphae with brush-like branching at 45° angles. The mycelial growth in pulmonary aspergillosis usually is not associated with the presence of conidiophores or fruiting heads, so that confusion with phycomycosis may

occur. However, fungi producing the latter disease are not septate (Figure 11). The presence of septate branching hyphae in cytologic material is strong morphologic evidence of infection. Cultures alone may be positive in the absence of true infection. Occasionally, sporeheads and mycelium may be seen. Aspergillus, along with several other organisms, has been implicated in the production of cellular atypias easily mistaken for squamous cell carcinoma[99,145,155,156] (Figure 12 and 13).

In Figure 14 is shown a hyphal fragment seen in bronchial washings from a leukemic patient with pulmonary phycomycosis. The correct diagnosis was made from this specimen. As can be seen from the illustration, the fungus is characterized by ribbon-like, nonseptate branching hyphae. They may vary widely in width from 6 to 50 μ. Culture is necessary to identify which fungus is causing the infection, as multiple organisms with identical tissue morphology may produce the disease.[155]

Parasites

In recent years the incidence of *Pneumocystis carinii* pneumonia has been increasing and is now recognized as occurring potentially in any situation of imparied immune response. More particularly, it is seen in infants who are premature or debilitated, in immunologic disorders, in immunoglobulin defects, in the presence of therapy with corticosteroids and chemotherapy, and in renal transplants. The introduction of effective therapeutic drugs has markedly increased the clinical importance of antemortem diagnosis.[157] On Papanicolaou-stained material, the organisms may be difficult to identify, as their staining is quite variable and faint, even in the most ideal of cases. Their most typical presentation on the Papanicolaou-stained smear is as a mass of partially eosinophilic or amorphous material. Within this mass may be a suggestion of small superimposed circlets (Figure 15, left). Although this presentation is not characteristic for pneumocystis, such a honeycombed, amorphous, eosinophilic mass should be further evaluated by special stains. In such a situation, one would decolorize this slide and restain with methenamine silver. This procedure immediately brings out the diagnostic features of these organisms. On methenamine silver staining, the organism is seen mainly as a spherical cyst measuring 6 to 8 μ in diameter (approximately that of an erythrocyte). Certain variations of this form can be seen. The organism can be cup shaped, crescent shaped, or crinkled. Depending upon the surface of the organism which is exposed to view, small globoid interior structures can be seen, some of which appear to be attached to the cyst wall (Figure 15, right and Figure 16). Some laboratories prefer a Giemsa stain for identification. With this technique one is able to identify

up to eight structures occurring within the cyst. These structures are about 0.5 to 1.0 μ in diameter, are easily overlooked, and may be confused with granules or cell fragments. Other organisms which must be considered in the differential diagnosis are candida species and histoplasma.

Although in the literature one can find reports of success in diagnosing this organism in sputum; tracheal aspirates; and washings from hypopharynx, bronchus, and stomach,[158-160] we generally have been unsuccessful in finding the organisms in such preparations. We have seen the organism most frequently in transthoracic thin needle aspirations, in bronchial brushing specimens, and in pulmonary lavage specimens. On only one occasion have we seen the organisms in sputum, and that sputum was a postbronchoscopy specimen.

In less than 2 years the authors have observed two pulmonary infections with the filariform larval stage of *Strongyloides stercoralis*. In both patients, prior disease was existent. One patient was a renal transplant recipient who had been supported with heavy steroid dosage. The second patient had an undifferentiated bronchogenic carcinoma. In both situations the primary diagnosis was suggested by cytologic examination of sputum. The organisms observed measured 400 to 500 μ in length and exhibited closed gullets and slightly notched tails (Figure 17). This morphology differentiated them from *Ascaris lumbricoides*, *Necator americanus*, and *Ancylostoma duodenale*.[145,155] One prior case of strongyloidiasis diagnosed in Papanicolaou-stained sputum smears has been reported by Kenney and Webber.[161] Other reports of the cytologic detection of parasitic pulmonary disease have included a report of pulmonary echinococcosis by Allen and Fullmer [162] and the lung fluke *Paragonimus kellicotti* by McCallum.[163]

Viral Infections

A number of laboratories have investigated the cellular changes in respiratory epithelium in response to a viral infection.[164-169] Particularly noteworthy here is the 1968 study of the cytologic features of viral respiratory tract infections by Naib and associates.[167] Cellular changes were noted in 41 of 99 patients with culture-proven viral infection. The specificity of the cellular changes was confirmed in 76% by conventional virologic procedures. Characteristic cellular changes were observed in patients with parainfluenza virus, adenovirus, and cytomegalovirus infections. The cellular changes in such patients can be divided into three general categories. First is that of a nonspecific cellular alteration characterized by ciliocytophthoria, first described by Papanicolaou in 1956.[170] This is a peculiar degeneration of the ciliated respiratory epithelium in

which a pinching off occurs between the cilia-bearing cytoplasm and the nucleated cytoplasm, leaving an anucleated mass of cytoplasm-bearing cilia and a degenerating nucleus and cytoplasm.[171,172] A more problematic type of nonspecific alteration is that of regeneration and atypia of the respiratory epithelium. This may present in sputum and bronchial material as tissue fragments composed of cells bearing enlarged hyperchromatic nuclei and enlarged prominent nucleoli.[99] A false positive diagnosis of cancer may be made if one is not knowledgeable of these changes and aware of a further possible etiology. The tightly coherent feature of the cells in the tissue fragments as well as the absence of atypical cells lying singly help in avoiding the diagnosis of cancer (Figure 18).

The more specific and diagnostic cellular alterations are of the most widely applied, practical significance in the presence of infections with herpes simplex virus and the cytomegalovirus. Changes with both of these infections are highly characteristic and have been well described in the literature.[165-169] The hallmark of cellular alteration produced by herpes simplex is that of cells with multiple, molded nuclei which may either contain eosinophilic, irregular inclusion bodies (Figure 19, left) or exhibit a peculiar type of nuclear degeneration which appears as slate gray homogenized nuclear contents (Figure 19, right). Cells infected by the cytomegalovirus are larger and may show some multinucleation, but they have fewer nuclei and none of the molding as seen in herpes simplex. Large, basophilic, smooth intranuclear inclusions surrounded by a very prominent halo and marked margination of chromatin on the inner surface of the nuclear membrane are present. Cytoplasmic inclusions also present in this disease may be manifested by the textured or "hammered aluminum" appearance to the cytoplasm (Figure 20).

Cytopathology of Primary Lung Cancer

Cytologic diagnosis of primary lung cancer may be made in conformity with the World Health Organization classification of lung tumors.[173] While from cytologic examination alone it is not yet possible to recognize some of the subtypes from sputum and bronchial material, it is possible to classify the tumors into the major categories: epidermoid carcinoma, with and without keratinization; large cell anaplastic carcinoma; small cell anaplastic carcinoma; and adenocarcinoma. The adenocarcinomas may be further subdivided from cytologic findings into those of bronchogenic acinar and papillary types and those of the bronchioloalveolar cell type. This method of cytologic reporting has two advantages: it requires the cytopathologist to consciously correlate cellular morphology with tissue morphology, and it alerts the cytopathologist to cell patterns that cannot

be classified into the histologic types of lung cancer. These latter cytologic patterns may indicate inadequate material for diagnosis, benign reactive respiratory tract disease, or metastatic cancer.

Epidermoid Carcinoma

In Saccomanno preparations of sputum, epidermoid carcinoma with keratinization is recognized cytologically by large, irregular, pleomorphic cells that are brightly orangeophilic when stained by the Papanicolaou method. The cytoplasm has a very heavy texture and refractile quality that can be accentuated by lowering the condenser of the microscope. The nuclei of these cells are as aberrant as the cells themselves. The chromatin is dense but, in well-preserved cells, is distributed in irregular clumps. Nucleoli are absent. The tumor cell population varies from a few cells to several hundred cells per smear. The diagnosis can be made in either case. The background may or may not exhibit a granular necrotic quality (tumor diathesis). If the background is inflammatory, caution in making a cancer diagnosis is necessary unless unequivocal tumor cells as described are seen (Figure 21). Cells of a dysplastic type may accompany keratinizing tumor cells. They are smaller counterparts of the keratinized malignant cells with less dense nuclear chromatin, less pleomorphism, and a lower intensity of cytoplasmic staining. While such cells may suggest a diagnosis of keratinizing squamous cell carcinoma, they alone may be seen in reactive and metaplastic lesions of the respiratory tract not necessarily related to carcinoma (Figures 12 and 22). Separation of these dysplastic cells between precancerous epithelial atypias and those that are merely benign reactive lesions is a formidable obstacle to lung cancer screening by cytology that is under intense investigation.[100–105,107,117,122–130,174–178]

Epidermoid carcinoma with keratinization of the lung may reach a large size and develop a central cavity of necrotic cells. Such a case may exfoliate large numbers of dysplastic cells with a strikingly necrotic background to the smear. A few unequivocal cancer cells are usually seen, and the overall pattern of the smear allows the cytopathologist to predict that a large cavitating tumor is present (Figure 23). Bronchial washings and brushings as well as fresh sputum samples produce the same cytopathology for this lesion as for keratinizing squamous cell carcinoma without cavitation.

Those epidermoid carcinomas with little or no keratin formation also have large pleomorphic cells with or without some dysplastic cells. Fresh and prefixed sputum have the same cytologic presentation with this tumor type, but both bronchial washings and bronchial brushings exhibit a

greater proportion of less differentiated cells and a greater degree of uniformity of the tumor cell population. In sputum, the tumor cells have a cyanophilic cytoplasm that has the same dense quality as that of the keratinized malignant tumor cells. The nuclear structure exhibits darkly staining, irregularly distributed chromatin. A rare tumor cell may contain a visible nucleolus (Figure 24). The number of tumor cells with nucleoli is greatly increased in bronchial washings and bronchial brushing specimens. Because of the presence of nucleoli the observer may be tempted to consider a diagnosis of adenocarcinoma; however, in contrast to the latter, many tumor cells do not cluster but occur in flat sheets with well-defined cell borders or predominantly as single cells with accentuation of the cytoplasmic membrane, giving the appearance of a double cell border. This double cell border, as illustrated in Figure 24, right panel, is a useful feature for identifying squamous cells in a relatively undifferentiated state in some of these tumors. Large clinical size of these tumors leads to degeneration and necrosis with consequent loss of cellular characteristics that specifically identify the tumor type in cytologic material.

Large Cell Anaplastic Carcinoma

Large cell anaplastic carcinoma usually provides many malignant cells for cytologic diagnosis. The characteristics of abnormal chromatin distribution, prominent nucleoli, and high nuclear/cytoplasmic ratio are marked. The most important differential observation in relation to the epidermoid carcinomas is the quality of the cytoplasm which is often finely vacuolated. The cytoplasmic borders are indistinct regardless of whether the cells are occurring in groups or as single cells. Clustering of cells is most frequently seen in bronchial washings and brushings, while a single cell pattern is most often seen in prefixed and fresh sputum (Figure 25). In the sputum cytology, the diagnostic difference between atypical reactive alveolar pneumocytes and cells of large cell cancer must be based on cells well enough preserved such that distinctly abnormal chromatin structure and prominent nucleoli can be seen. An irregular nuclear membrane is also seen, but lobulation of the nucleus, a feature of cells of adenocarcinoma, should be infrequent in the cytology of the large cell anaplastic carcinomas. If degeneration and necrosis occur to a significant degree, the sputum cytology will consist of small degenerated cells in loose arrangement and surrounded by a necrotic background. The pattern will suggest a diagnosis of small cell anaplastic carcinoma, but careful examination will reveal a few tumor cells that are too large for that diagnosis (Figure 26).

Small Cell Anaplastic Carcinoma

The most significant differences in cell morphology dependent upon the type of cytologic preparation occur with small cell anaplastic carcinoma. Sputum prepared by the Saccomanno technic reveals loose clusters of small malignant cells that have a "blown apart" appearance on the smear. The nuclei are markedly hyperchromatic, average about one and one-half times the diameter of lymphocytes, and are very irregular in outline. Many cells are devoid of cytoplasm, while others have only a small wisp of cyanophilic cytoplasm that blends in with granular material, making up a tumor diathesis background. The quality of this background, made up largely of degenerating cytoplasmic material, reflects the necrosis that is commonly seen with the histopathology of this tumor. Close examination of the nucleus of these tumor cells may reveal blocks of irregular chromatin and occasionally a nucleolus. Except for the areas that reveal tumor cells, the background of the rest of the smear may be remarkably clean and contain few or no histiocytes. Some sputum smears from small cell carcinomas are very inflammatory. This tends to disperse and obscure the tumor cells, making both screening and correct diagnosis difficult.

Bronchial washings (Figure 27) and fresh sputum (Figure 28) from cases of small cell carcinoma present tumor cells that are much more cohesive. Their individual cell morphology is as described previously, but the cohesiveness of the groups with actual nuclear molding and compression makes examination for nuclear detail difficult and accentuates the hyperchromasia. The screener and the cytopathologist must, therefore, rely on the degree of nuclear compression and molding as a major diagnostic criterion. Bronchial brushings show this nuclear molding to an even greater extent. Cell size in comparison to sputum and bronchial washings is the most important difference seen with small cell anaplastic carcinomas in bronchial brushing material. The tumor cells appear to be twice as large in bronchial brushings as the same tumor cells in a prefixed or fresh sputum and give little support to the frequently voiced opinion that the cells of small cell carcinomas are about the same size as lymphocytes.

Adenocarcinoma

The cells of adenocarcinoma of the lung provide a variety of cytologic patterns for analysis. A number of reactive conditions within the respiratory tract may mimic adenocarcinoma. In analyzing cytologic specimens, both subjective and objective criteria have been used to diagnose and separate various types of adenocarcinoma of the lung.[179] The results of these studies indicate significant differences in cytologic features. These include numbers of cells per smear, depth of focus of cell groups, spatial

arrangement of cells within groups, nuclear and cytoplasmic structure, and staining. These may be used to classify adenocarcinoma of the lung according to the World Health Organization classification of lung tumors.

In both sputum and bronchial washing specimens, bronchioloalveolar cell carcinoma usually provides the largest number of cells usually seen with any of the adenocarcinomas. The only exception to this is the clinical presentation of bronchioloalveolar cell carcinoma as a small peripheral mass. The cells occur both singly and in groups, but observations of the spatial arrangement and depth of focus of the groups lead to the correct diagnosis. Individual tumor cells are uniform round to oval, with uniform round nuclei and small round nucleoli. Chromatin is finely granular and almost bland in well-preserved material. In the cellular groups, a broad range of focusing is required to see all of the cells clearly. The examiner also notes that each cell within the group, while attached to the others, retains its independent shape (Figure 29). The cytoplasm of these tumor cells may be clear or finely vacuolated. In Saccomanno-type preparations, it is quite rare for cells of bronchioloalveolar cell carcinoma to have sharply bordered vacuoles (Figure 30).

In smears from fresh sputum the cytologic presentation of bronchioloalveolar cell carcinoma is similar to that seen in prefixed material; however, there is one striking difference. In fresh material, vacuolated cytoplasm is frequently noted. In a recent review of 21 consecutive, histologically confirmed cases, in which specimens were prepared from fresh sputum, vacuolated cytoplasm was noted in 9 (Figure 31). Of these 9, 4 had vacuoles of the large hyperdistended type. Many of these vacuoles contained well-preserved polymorphonuclear leukocytes. The reasons for this variant in the presentation are not known at this time. One possibility is that the vacuoles are fragile and are destroyed by the chemicals used in the Saccomanno technique. Regardless of the type of preparation, however, the cells still retain independent configuration within the cell group. Occasionally, psammoma bodies may be seen in association with these tumors.[179,180]

Bronchial brushing specimens present the same cell configurations and individual characteristics described, including the appearance of abundant histiocytes in the background of the smears. This technique makes possible the diagnosis of bronchioloalveolar carcinoma in its clinical presentation of the small peripheral mass.

Those adenocarcinomas arising from bronchial glands and surface columnar epithelium may grow in acinar, papillary, or combined patterns. In both bronchogenic papillary and bronchogenic acinar carcinomas, the number of tumor cells in sputum and bronchial washings is much less

than in bronchioloalveolar cell cancers. The cytology of bronchogenic papillary tumors reveals features that are quite similar to bronchioloalveolar cell carcinomas; however, there is somewhat less depth of focus to the cell clusters and more irregularity of nuclear chromatin structure. Nucleoli are more prominent (Figure 32). Histologically such tumors may show cells that are highly vacuolated. As has been described for fresh sputum smears of bronchioloalveolar cell carcinomas, similar preparations from acinar tumors may show vacuoles.

Cases of bronchogenic acinar adenocarcinoma reveal clusters of tumor cells with less depth of focus than bronchogenic papillary or bronchioloalveolar cell carcinoma. The cells and nuclei definitely overlap and do not occur in flat sheets. The nuclei are larger than in the other two types of adenocarcinoma described, although an actual difference in nuclear/cytoplasmic ratio could not be demonstrated by cell measurements.[179] Cell borders are blurred, giving the impression of a syncytium of tumor cells. Variation in differentiation of bronchogenic acinar carcinomas affects the cytologic presentation, as does the type of specimen. Poorly differentiated adenocarcinomas of acinar type have eosinophilic cytoplasm in prefixed sputum. The same tumor in fresh sputum, bronchial washings, and brushings will have cyanophilic cytoplasm. The cytoplasm will also be more intact in the latter types of specimens, while in sputum it is fragmented, creating the tumor diathesis background. Because of the relative pleomorphism of tumor cells in poorly differentiated bronchogenic acinar carcinomas and the eosinophilic cytoplasm, there is a tendency to report such cases as squamous cell carcinoma. The feathery quality to the cytoplasm of the tumor cells and the lack of definite cell borders with the clustering of nuclei should always point toward the correct diagnosis of adenocarcinoma (Figure 33).

Well-differentiated bronchogenic acinar carcinomas have tumor cells with cyanophilic cytoplasm in all types of specimens. Clustering of nuclei and relatively indistinct cell borders are the clues to the correct diagnosis of adenocarcinoma. Nucleoli are prominent, and occasional actual acini may be seen in bronchial washings or brushings. The cytoplasm is finely vacuolated or homogeneous. Sharply bordered vacuoles may be seen in fresh material.

The variation in cytoplasmic staining with the adenocarcinomas, particularly those of bronchogenic acinar type, is probably as much a reflection of cell preservation as differentiation of the tumor. Koss was the first to note this.[99] It occurs with other types of lung cancer if poor preservation of the specimen is extreme. Direct sampling techniques give the most re-

liable staining and diagnostic features of tumor cells but are impractical if screening for lung cancer becomes a reality.

Giant Cell Carcinoma

The basic cellular pattern of presentation in giant cell carcinoma is as some form of adenocarcinoma, usually a poorly differentiated one.[181-183] Neoplastic giant cells, often multinucleate, are present in most but not all cases. These giant cells average two to three times the size of the other malignant cells in the smears. Their nuclear structure and other features are magnified examples of the basic underlying tumor cell pattern, be it adenocarcinoma or, rarely, poorly differentiated squamous cell carcinoma (Figure 34). The authors have found that the only confusing differential diagnostic problem in the cytologic presentation of a giant cell carcinoma may be its simulation by carcinoma metastatic to a major bronchus. The unusually large numbers of tumor cells and their pleomorphism present a picture identical to the cytology of a primary giant cell carcinoma of the lung.

Mucoepidermoid Carcinoma

The remaining specific type of lung cancer that can be recognized cytologically is the combination of squamous cell carcinoma and adenocarcinoma (mucoepidermoid carcinoma). The dominant feature on cytologic smears is that of adenocarcinoma. The epidermoid features are found in scattered, isolated cells and are easily overlooked. These cells are usually poorly differentiated, and their cytoplasm is cyanophilic. The cytoplasmic staining of the cells is characteristic of adenocarcinoma. Examination of the cell borders and density of the cytoplasm will reveal differences between the squamous cells and glandular cells, the former having a dense quality to the cytoplasm and a double border to the cell outline. Nucleoli may be present in both types of cells but are more frequent in the adenocarcinoma cells. The adenocarcinoma component will also cluster as in any case of adenocarcinoma, but isolated malignant squamous cells may be attached to these same clusters (Figure 35). It is the differences in morphology of these single cells in an otherwise homogeneous group of cells that may be the only clue to the correct cytologic diagnosis of mucoepidermoid carcinoma of the lung. Both elements must be clearly present histologically in such a tumor to be recognized from cytologic material. The cases of poorly differentiated squamous cell carcinoma with scattered mucicarmine-positive cells are not considered mucoepidermoid cancers in the cytologic sense.

Other Neoplasms

In addition to the frequently encountered cytologic presentation of the various types of bronchogenic carcinoma, other less common primary neoplasms presenting cytologically have been described. These have included five different reports of Hodgkins disease,[184-188] and one study dealing with pulmonary lymphoma in general.[189] Other neoplasms reported have included papilloma,[190] leiomyosarcoma,[191] malignant histiocytoma,[192] and bronchial adenoma.[193]

Cytopathology of Metastatic Cancer to the Lung

The cytopathologist must be aware that cancers of adjacent sites (esophagus, oral cavity) as well as tumors metastatic to the lung may exfoliate tumor cells into sputum or bronchial washings.[194-199] As stated by Koss, approximately 50% of metastatic tumors to the lungs may be diagnosed cytologically.[99] Bronchial brushings may directly sample metastatic tumors to the lung. In cancers from adjacent sites there are usually few tumor cells. Squamous cell cancer makes up the majority of lesions of the oral cavity and esophagus that contaminate sputum specimens with tumor cells. In practically all cases the cells are small, round, and parabasal-like in configuration. Such cells may strongly resemble those exfoliating from bronchial dysplasias. They occur singly or in small clusters. In the cases of primary squamous cell carcinoma of the esophagus, the cells are rarely seen unless there is a tracheoesophageal fistula. In that situation there is an intense inflammatory background. This feature plus these small dysplastic cells make exact interpretation of the case as representing any type of carcinoma extremely difficult.

The importance of recognizing specific cytologic patterns of lung cancer leads the cytopathologist to the observation of unusual cell patterns where malignant cells are definitely present in sputum or bronchial washings. Such cases not infrequently represent cancer metastatic to the lung. Metastatic tumors to the lung occurring as diffuse nodules or as single tumors involving a major bronchus present different cytologic patterns. In cases of diffuse metastasis, tumor cells occur in clusters simulating a pattern of bronchioloalveolar cell cancer but with far fewer cells, perhaps only five to ten such groups per smear. The background of the smear is clean, with virtually no histiocytes. Recognition of the primary site is possible in some cases by noting cell characteristics and spatial arrangement. Thus, breast cancers of the duct type produce cells with pale chromatin and prominent nucleoli. The cells show little depth of focus and occur in relatively tight clusters with conspicious nuclear molding. Large, highly vacuolated and pleomorphic cells are seen in metastases

from pancreatic and ovarian cancer. If prior surgical tissue is present for comparison with the cytology, it is at least possible to say that the tumor cells are consistent with the previous primary cancer. Differences noted between the cytologic specimen and the tissue specimen may signal the presence of a second cancer that is either metastatic to the lung or a primary cancer.

Comparison between the histology and sputum cytology of a primary cancer is probably most important where metastasis has occurred as a single mass to a major bronchus. Clinically and radiographically these lesions simulate primary lung cancer and they produce abundant tumor cells in sputum and bronchial washings in a pattern that also may resemble primary lung cancer. However, there is a greater degree of pleomorphism of tumor cells than usually is seen in primary lung cancer, except for giant cell carcinoma. In the experience of the authors, metastatic carcinoma of the colon is the tumor that cytologically may suggest a giant cell carcinoma of the lung. The cells in the metastatic carcinoma of the colon are much larger and more intensely eosinophilic in cytoplasmic staining than primary adenocarcinomas of the lung. Other tumors metastatic to the lungs which the authors have encountered in their laboratories have originated in stomach, liver, kidney, prostate, bladder, testis, uterus, and also have included Hodgkin's disease, lymphomas, leukemias, sarcomas, and malignant melanomas.

Cytopathology of Early Lung Cancer

Control of the lung cancer problem would seem to depend on detection of early cases. Saccomanno *et al.* have described the developmental sequence of cytologic changes in the evolution of epidermoid carcinoma of the lung. They estimate an average time of existence of 5 years each at the three phases: severe dysplasia, carcinoma *in situ*, and microinvasive carcinoma.[128,130] These investigators have also noted the remarkable individual variation in cytologic changes to lung irritants, smoking and uranium exposure in mining being two examples.[107] Histologic correlation of precancerous lesions of the bronchus with smoking habits was described by Auerbach *et al.* 18 years ago.[123,126] Other investigators have confirmed this association,[124,200] but a few have doubted it.[103,125] These latter studies have not been as exhaustive as the studies of Auerbach. Auerbach *et al.* have been able to reproduce the same histologic sequence of developing epidermoid carcinoma of the lung in smoking beagle dogs,[175] while Schreiber and co-workers have induced the same type of carcinoma in hamsters using benzo[*a*]pyrene-ferric oxide.[176,177] These workers have followed the cytology of the development of squamous cancers in this

experimental model and find it quite similar to that in male cigarette smokers and uranium miners.[123,176,177]

Papanicolaou and Koprowska were the first to report the cytologic detection and localization of a carcinoma *in situ* of the lung.[30] A number of subsequent reports have appeared indicating that cytology is quite accurate in this stage of lung cancer of the squamous type and that localization of the lesion can be accomplished.[201-209] Survival has been greatly improved in this highly select group of patients. This experience is very favorable when compared to screening for lung cancer by routine periodic chest x-rays.[210]

Armed with these preliminary data, the National Cancer Institute has funded projects for the detection of early lung cancer at the Mayo Clinic, The Memorial Center in New York, and Johns Hopkins Hospital. While the various approaches are slightly different, screening is aimed at male, heavy cigarette smokers over 45 years of age. There is a control and test group, the latter receiving intensive periodic cytologic screening of sputum as well as chest films. As of July 1975, the Mayo project had screened 8361 high-risk subjects with a initial detection of 60 unsuspected primary lung cancers. This is a prevalence rate of 0.9%. The calculated incidence rate in the test group is 4.8 cases/thousand man-years of surveillance. Both the prevalence and incidence rates are lower than expected and make routine screening of *unselected* populations of smokers unrewarding. Localization of these early tumors has been accomplished by systematic fiberoptic bronchoscopy and brushing of all possible bronchial segments. Obviously, this is a time-consuming and expensive procedure. While screening of such material is easy, and one can readily detect abnormal cells against a background of normal bronchial cells, the costs per patient and treatment methodology pose problems. The documented multicentricity of lung cancers, which has not been a factor previously in the clinical management of lung cancer, now becomes of importance and has dictated conservative surgical treatment in the Mayo Clinic cases and, recently, the use of intrabronchial cryotherapy.[207,208,211]

The cytologic description of developing squamous cell carcinoma has focused on metaplastic and dysplastic cells of squamous type that are similar to those seen in cervicovaginal cytology. While such cells may occur as nonspecific reaction to lung irritants, their progressive increase in number and degree of atypia (seen principally as an alteration of nuclear chromatin structure and predominance of dense orangeophilic cytoplasmic staining in any given patient) indicates that epidermoid carcinoma is developing. At what point an actual, histologically acceptable carcinoma *in situ* which can be diagnosed by cytology becomes present is

not entirely clear from the cases studied to date. Usually a large number of very dysplastic orangeophilic squamous cells are described with a few malignant tumor cells that would be usually associated with invasive squamous cell carcinoma (Figures 36). The authors feel that the dysplastic squamous cell has been overemphasized. Small parabasal-like cells, with high nuclear/cytoplasmic ratios and very granular nuclear chromatin, occur in conjunction with the dysplastic cells. The presence of these in the sputum cytology seems to strongly indicate the presence of an *in situ* epidermoid carcinoma of the lung (Figure 37). The similarity of these cells to those seen with *in situ* carcinoma of the cervix is striking, but they seldom occur in syncytial arrangement in sputum.

Screening and Diagnostic Accuracy of Respiratory Cytopathology

At the heart of any study in respiratory cytopathology lies the basic issue of its relevance to diagnosis and treatment in clinical disease. In what percentage of patients with lung cancer can cytology detect its presence? To what extent can cytology correctly predict the histologic classification? This section of this review addresses these two questions. Answers to them have been sought through many studies.[212-234] In the study by Koss and his associates of 149 patients with histologically proven lung cancer, the overall accuracy of cytology in detecting the presence of the tumor was 89% when three or more cytologic specimens were examined.[222] The importance of multiplicity of specimen examination was amplified by the 1970 study of Erozan and Frost. Of 107 patients with lung cancer, one bronchoscopic examination yielded diagnostic cytology in 61% of patients, while one sputum specimen was diagnostic in only 42% of 141 cases. However, diagnostic pickup increased to 82% with three sputum specimens and to 91% with five.[227]

In general, among proficient cytopathology laboratories, screening of sputum for lung cancer will detect about 80% of the cases. Accuracy is very high for central tumors unless there is significant bronchial obstruction, but this is balanced by less favorable results for peripheral tumors that are usually asymptomatic. Bronchial brushing techniques for peripheral lesions [235-249] have improved diagnostic accuracy in cancer detection in the range of 70 to 80% of cases [250-251] Hattori *et al.*, using fluoroscopic control of brushing, have achieved diagnosis in 83% of peripheral lung cancers.[252-255] More importantly, brushing can reach the asymptomatic lesion, revealing a diagnosis of cancer in more favorable situations for cure and thus improve survival. Routine sputum cytologic diagnosis of lung cancer in the usual clinical cases, through accurate, fails to improve survival.[256] This latter report documenting survival in relation to cytologic diagnosis

of lung cancer has emphasized the necessity for screening the asymptomatic high-risk groups.

The authors have stressed not only screening accuracy in detecting cancer of the respiratory tract but accuracy in reporting the correct histologic type of cancer from the cytology. This latter policy has proven itself to be a most informative method of reporting, for maintaining quality control in the cytopathology laboratory, and an important concept in control of false positive diagnosis of cancer. Cytologic smear patterns that fail to conform to recognizable pattern of lung cancer frequently are the result of benign reactive conditions within the respiratory tract or metastatic tumors to the lung. Many of the latter can be recognized for what they are because of the presentation of malignant cells in patterns different from those seen in primary lung cancer cases. Table 1 shows the results at the Medical College of Virginia of attempting to classify lung cancer from its cytologic presentation. During the period of study, 1970 through 1974, overall accuracy in diagnosing lung cancer by cytology was 94.4% in those patients having one or more specimens when the diagnosis was made or suggested. The results of classification are excellent for the major lung cancer types, specifically in relation to adenocarcinomas which may be quite difficult to diagnose. The poor results with undifferentiated large cell carcinomas reflect the variability of this tumor as seen cytologically. The screener and the cytopathologist in many of these cases attempt to classify the lesion into one of the more common types. This can be seen in the spread of diagnoses that were made. Difficulty has been experienced in recognizing the mixed carcinomas because in histologically

Table 1—Prediction of Histology of Lung Cancer from Cytologic Material in 310 Patients at the Medical College of Virginia

	Cytologic diagnosis					
Histologic diagnosis	Squamous cell carcinoma	Large cell undifferentiated carcinoma	Small cell undifferentiated carcinoma	Adenocarcinoma	Mucoepidermoid carcinoma	Percent correlation
Squamous cell carcinoma	171	2	3	7	1	92
Large cell undifferentiated carcinoma	5	10	6	3	0	41
Small cell undifferentiated carcinoma	1	1	48	4	0	88
Adenocarcinoma	4	0	0	32	1	86
Mucoepidermoid carcinoma	5	1	0	5	0	0

diagnosable form they are uncommon and cytologically the predominant pattern is usually adenocarcinoma with few recognizable malignant squamous cells. During the same 5 years of this study there were 15 false positive diagnoses for respiratory tract cancer. This is a false positive rate of 4.8% calculated against proven cancer diagnosis by histology. If clinical lung cancers are included, the rate is 3.4%, and if calculated in terms of the total respiratory tract specimens handled during the same period, the rate is 0.16%.

At Duke University, during the 5-year period of 1970–1974 529 histologically confirmed lung cancer patients were seen. Of these, 420 patients had one or more satisfactory cytologic specimens examined. In 253 patients a cytologic interpretation diagnostic of cancer or suspicious of cancer was given. This is an overall detection rate of 60.2% (253 patients out of 420) and is a true reflection of the reality of respiratory cytopathology as it actually contributed to diagnosis in these patients. In those from whom only one specimen was obtained, cytologic detection was only 41.9%, but cytologic detection was increased by 30% when three or more cytologic specimens were examined. Table 2 further reveals the significance of multiplicity of specimens in lung cancer diagnosis. As is shown of lung cancers which can be detected by cytology, five specimens will yield a detection rate of greater than 95%. In this Duke series, a definite cytologic cancer diagnosis with prediction of tumor type was made in 226 patients. The correlations with the histology are shown in Table 3.

During this same period (1970–1974), cytologic specimens from 378 patients were interpreted as diagnostic or suspicious of cancer. A confirmatory tissue diagnosis was obtained in 253 patients. In 117 patients, treatment was begun without tissue confirmation, a procedure which is gaining evergrowing acceptance throughout the country. *The authors would recommend this only in those situations in which the highest quality of respiratory cytopathology practice is available.*

Seven false positive cytologic diagnoses were made at Duke during the

Table 2—The Significance of Multiplicity of Specimens in the Cytologic Diagnosis of 253 Histologically Confirmed Lung Cancers Detected by Cytology

No. of specimens	Cumulative No. of cytologic diagnoses of cancer	Percent of total 253 patients
1	158	60.4
2	198	78.1
3	221	87.3
4	236	93.2
5	245	96.6

Table 3—Prediction of Histology of Lung Cancer From Cytologic Material in 226 Patients at Duke University

Histologic diagnosis	Cytologic diagnosis					
	Squamous cell carcinoma	Large cell undifferentiated carcinoma	Small cell undifferentiated carcinoma	Adenocarcinoma	Mucoepidermoid carcinoma	Percent correlation
Squamous cell carcinoma	86	23	0	4	2	74.8
Large cell undifferentiated carcinoma	5	24	0	4	2	68.5
Small cell undifferentiated carcinoma	0	1	28	1	0	93.3
Adenocarcinoma	0	7	0	35	0	83.3
Mucoepidermoid carcinoma	0	1	0	0	3	75

period of this study. As computed against histologically proven cancer, this is 2.7%. Including the clinical cancers treated from cytology alone, the rate falls to 1.8% of all lung cancers diagnosed cytologically. When expressed in terms of total respiratory specimens examined (9892), the false positive rate is 0.07%.

A few incorrect cytologic diagnoses of respiratory tract cancer will occur in any cytopathology laboratory that tries to achieve maximum screening and diagnostic results from pulmonary cytology specimens. Most of these incorrect diagnoses will be the result of atypical metaplastic and proliferative responses. These responses seen with benign conditions have been documented by a number of authors.[116-118,122,131] Some have considered these changes precancerous,[257] while others doubt this relationship.[116] These reactive changes can be grouped into squamous metaplasia and dysplasia and bronchial and bronchioloalveolar hyperplasia and dysplasia both histopathologically and cytopathologically. Examples of the problems are seen in Figures 38 and 39. In the case of squamous metaplastic and dysplastic changes, the cytopathologist's problem is one of determining the levels of individual cellular abnormality that permit a differentiating between carcinoma and the benign reaction. Although there is the same problem in cervicovaginal cytology, with bronchial and bronchioloalveolar proliferations there is much less quantitative difference, particularly with brushing specimens. The emphasis is then on subtle differences in nuclear structure and spatial arrangement of cells in groups, as seen in Figure 40, which compares a bronchioloalveolar cell carcinoma with a pulmonary infarct.

Applications of Electron Microscopy to Respiratory Cytology

Although as yet there are still few papers published which deal with electron microscopy of pulmonary cytologic material,[258-263] there is a growing interest in laboratories everywhere in the valuable information to be gleaned from such studies. The current status of many aspects of this area have been summarized by Kory.[264] While it has not been useful as an aid in differentiating between benign and malignant cells, its applications in revealing ultrastructural characteristics of cellular differentiation have been striking. It has also been utilized in virologic studies.[260] Two ultrastructural studies performed on bronchioloalveolar cell carcinomas in our laboratories are illustrative of the great value of this technique when used in conjunction with conventional light microscopy.

The first study reported the use of electron microscopy to augment the routine cytopathologic study of cells in cerebrospinal fluid. The material for study consisted of multiple specimens of cerebrospinal fluid taken from a 24-year-old white female. The patient later died after a 9-month illness characterized by multiple bizarre neurologic symptoms. Light microscopy revealed a homogeneous population of cells, obviously malignant, but of uncertain derivation. Electron microscopic study showed prominent microvilli, numerous mitochondria, multiple foci of well-developed Golgi apparatus, several types of lysosomes, and one annulate lamella. The ultrastructure of these cells resembled that which has been described for Type 2 alveolar cells from the lung. Autopsy revealed alveolar cell carcinoma of the left lung with widespread metastases, including massive involvement of the leptomeninges.[261]

In the second study the material consisted of specimens of sputum taken from a 75-year-old white woman. The patient later died 3 months after the beginning of radiotherapy for alveolar cell carcinoma of the lung. Light microscopy revealed a homogeneous population of cells, obviously malignant, and interpreted as being derived from alveolar cell carcinoma. Electron microscopic study showed prominent microvilli, numerous mitochondria, multiple foci of well-developed Golgi apparatus, and several types of lysosomes. The ultrastructure of these cells resembled that which has been described for Type 2 alveolar cells from the lung.[263]

Thus, in these two studies, ultrastructural cellular characteristics permitted the augmentation of a light microscopic diagnosis of cancer with an electron microscopic diagnosis of cancer type and tissue of origin.

Cell attachment is of particular importance in both cytopathology and in studying the biology of cancer cells. The importance of spatial arrangement of cells in respiratory cytology has already been discussed. The

actual ultrastructure of these arrangements has yet to be studied. Unfortunately, it is these cell attachments that provide one of the major obstacles to automated cytology screening systems.

Automated Cytology Screening

Investigations to develop an automated system for cytology screening have been in progress for some time. Such systems seem to be the only feasible method for screening the female population at risk for developing uterine cancer. Lower than expected incidence and prevalence rates for lung cancer from the early lung cancer screening projects also make some method of automated cytology necessary to screen at practical and reasonable cost even a selected high-risk group of patients.

Two types of automated systems are being investigated at the present time: static systems, in which the cells are dispersed but fixed to slides of film, and flow systems, in which the cells move as single cells through a capillary type of aperture for measurement. The most sophisticated static systems involve image processing of the cells. Wied *et al.* have developed a highly accurate image processing system, Taxonomic Intra-Cellular Analytic System (TICAS) using computer analysis of multiple measurements of ultraviolet light (UV) absorption pattern of the cell.[265-267] Using this analytic system on known uniform populations of cells and storing the date recorded, the system can distinguish different cell types in a mixed population of cells as seen in a cervical vaginal smear. The accuracy of the system has been demonstrated. Unfortunately at present, it is a time-consuming methodology and is very expensive on a cost per case basis.[268,269]

Flow systems use one or two parameters of UV absorption, transmission, fluorescence, and more recently, light scattering to separate cells into normal and abnormal types. While the methods are less exacting than the most detailed image processing systems, they are more economical and faster. Both systems must have a low false negative rate and reasonable false positive rate to be practical in the laboratory setting. Wheeless *et al.* have developed a slit scan technique—initially on static specimens, but recently within a flow system—using an argon ion laser to excite fluorescence.[270] There are problems that are inherent in flow systems: presence of clumps of cells and mucus material, binucleate cells, orientation of cells in the stream of flow, staining of cells in suspension, and compilation of a significant data base from slit scan studies on known populations of cells.

Wheeless *et al.* and others are investigating all of these problems. Mechanical methods, such as syringing, may be the most useful method for cell dispersal without cell loss.[271] Centrifugal dispersal seems less

practical and satisfactory.[272] The mechanical blending method of Saccomanno for sputum may be adapted to automated cytology since it does disperse cells without significant cell loss.[273] A variety of enzymes and chemicals are being investigated to dissociate cells from mucus and to dissolve the desmosomes.[274] Preliminary results are not favorable.

Flow systems with one exception depend upon staining of cells in suspension by fluorochromes. Effort is being directed to find specific fluorescent markers that would identify cancer cells. A fluorochrome with a narrow emission spectrum that can be coupled to a specific cell marker is needed. The chemistry of conjugating such a substance with a specific antibody for use as a tumor cell tag is complex. The development of specific antibodies to tumor cells has also proved very challenging. Antibodies to cervical cancer cells have been produced that are reasonably specific, but they have a low titer and large quantities of both tumor and serum from immunized rabbits are required for absorption and final production of antibody.[275,276]

One interesting static and flow system that is undergoing further experimentation and refinement depends on the ability of unstained cells to scatter light. The scattered light is measured at thirty-two different angles, and the intensity coupled with the angle is recorded by a computer. From the static system, a Fourier transformation is produced from photographic images of cells and appears to be different for different types of cells.[277,278] In the flow system, a mathematical clustering algorithm is used to separate cells into classes, and a linear separation algorithm is used to determine the boundaries of the classes.[279] The technology has been used for military target analysis of data from high altitude spy satellites.

In contrast to image processing systems where the cells are preserved in a fixed retrievable state on film or slides and can be viewed by the light microscope, the flow systems do not allow for direct viewing after measurement analysis. To overcome this defect, and to document what the flow system is actually looking at and measuring, sophisticated sorting devices are being built. Those for the light scattering techniques are physically sorting the cells after the measurement based on computer analysis of differences in signal patterns. The slit scan technique may depend on development of a stop-action photographic system for viewing the cells within the flow at the time of measurement.

While the basic effort in automated cytology is directed to the analysis of cervicovaginal specimens, it seems reasonable that the technology will be applied to respiratory as well as other types of cytology specimens. Investigations so far, while they have not produced a commercially available or practical system, have significantly furthered the knowledge of cell

biology. Much of the credit and stimulus toward these studies must rest with the original report of the Papanicolaou smear as the fundamental cytologic technique for the detection of a cancer, a clinical and cytopathologic discipline that is less than 50 years old.

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Acknowledgments

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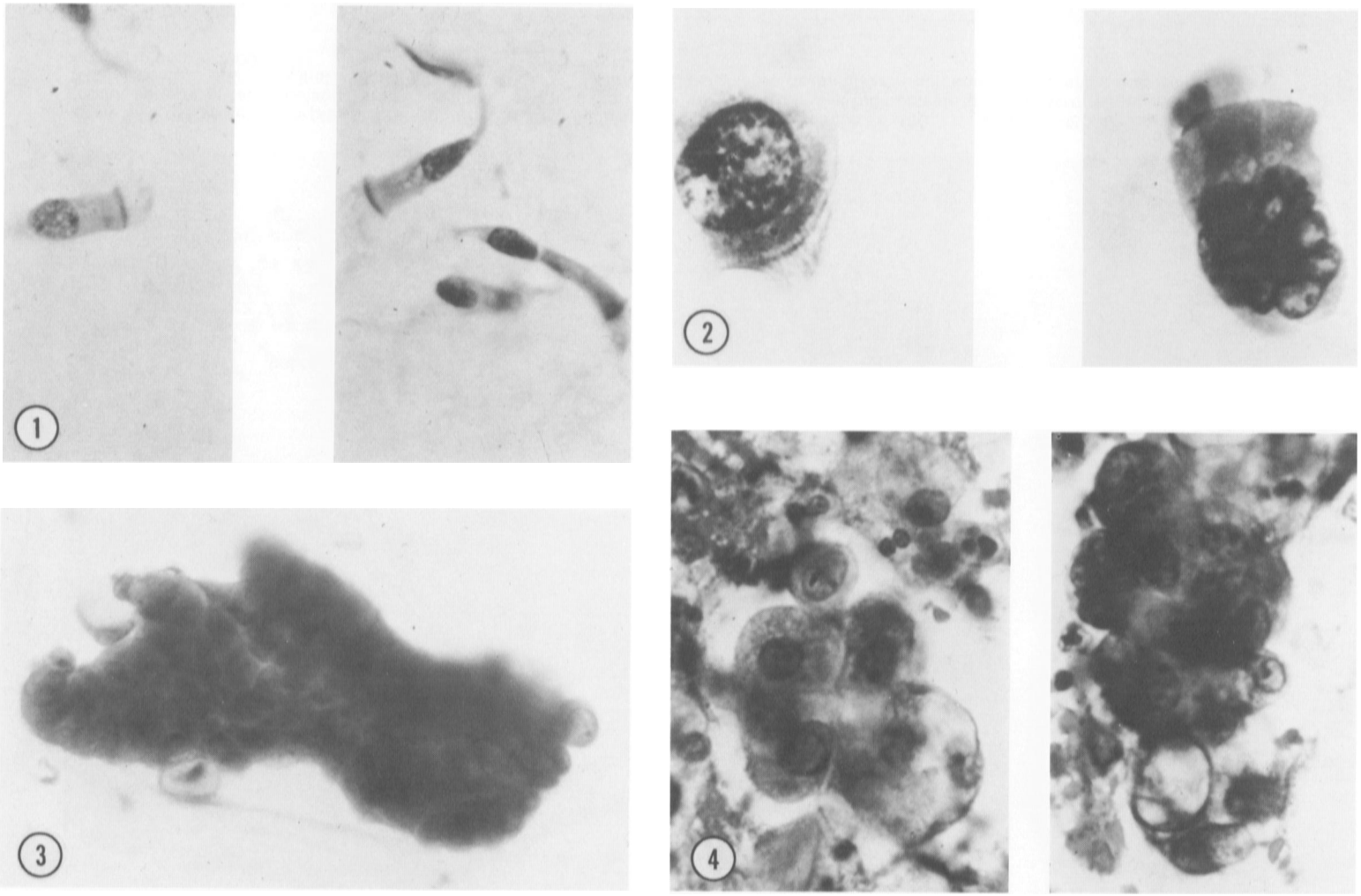
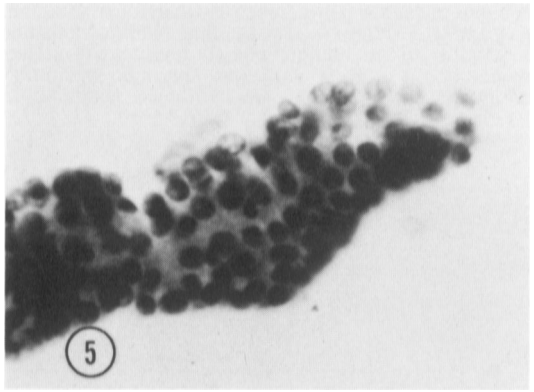
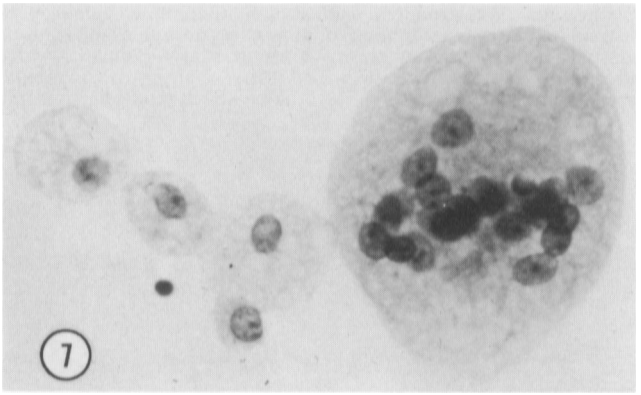
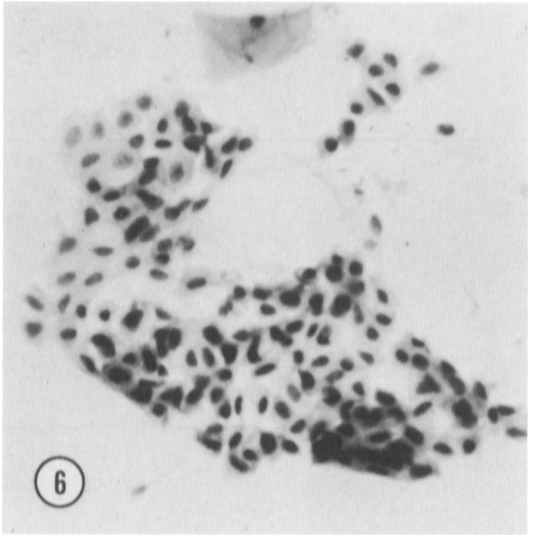


Figure 1—Bronchial columnar cells; bronchial washing specimen (Papanicolaou stain, original magnification × 1000). **Figure 2 (left panel)**—Single irritated bronchial columnar cell; fresh sputum (Papanicolaou stain, original magnification × 1000). **(right panel)**—Multinucleate irritated bronchial columnar cell; fresh sputum (Papanicolaou stain, original magnification × 1000). **Figure 3**—Hyperplasia of bronchial epithelium; fresh sputum (Papanicolaou stain, original magnification × 400). **Figure 4**—Reactive alveolar cells, pulmonary infarct; fresh sputum specimen. Obviously, many of the features of adenocarcinoma are present, and it is easy to make such a diagnostic error. Clinically, such patients may have a lung mass which radiologically may not look like a pulmonary infarct. Though these are large cells, note that the nuclear cytoplasmic ratio is nearly normal and there is marked variation in vacuolization of the cytoplasm. (Papanicolaou stain, original magnification × 1000)







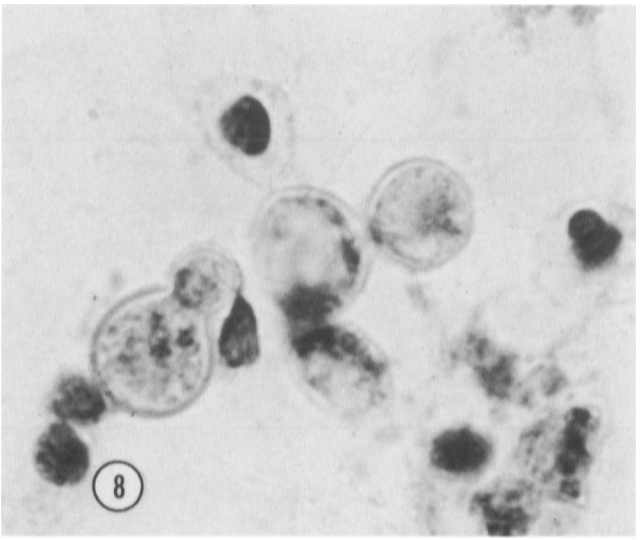
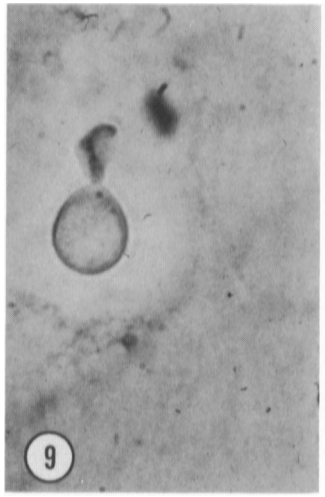
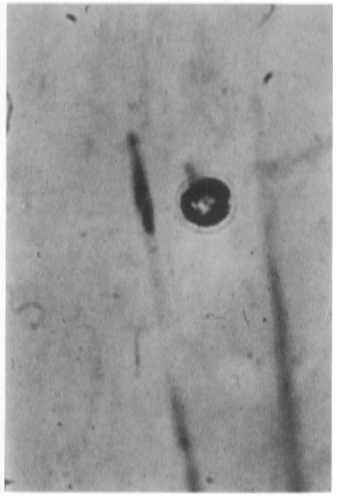
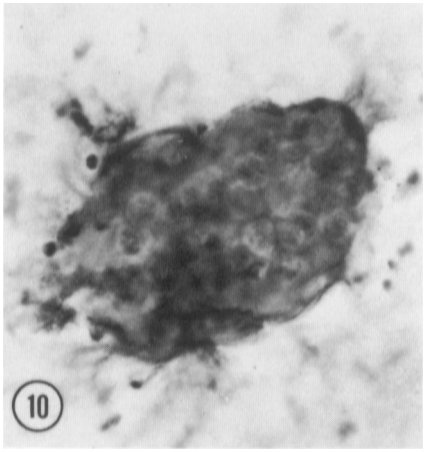
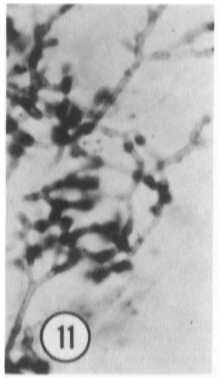


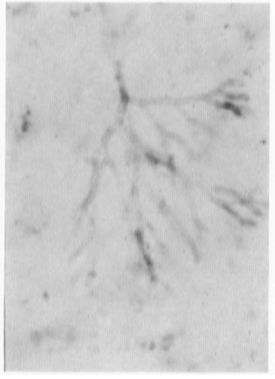
Figure 5—Reserve cell hyperplasia; fresh sputum (Papanicolaou stain, original magnification × 400). **Figure 6**—Squamous metaplasia; fresh sputum (Papanicolaou stain, original magnification × 400). **Figure 7**—Multinucleate giant macrophage; fresh sputum (Papanicolaou stain, original magnification × 400). **Figure 8**—*Blastomyces dermatitidis;* fresh sputum (Papanicolaou stain, original magnification × 1000).

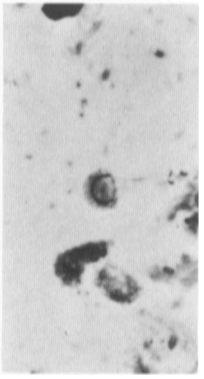


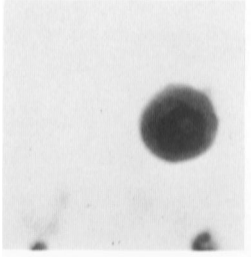


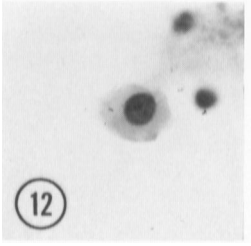












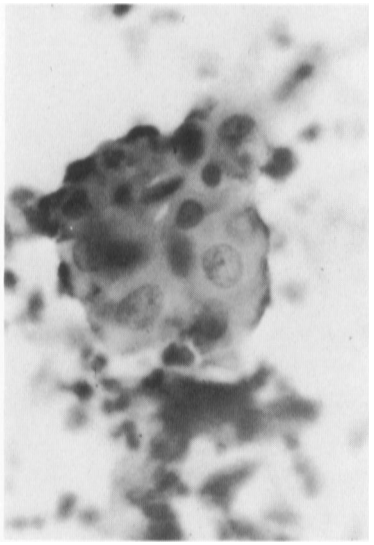
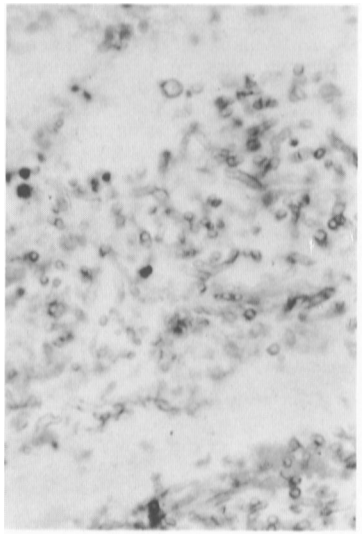
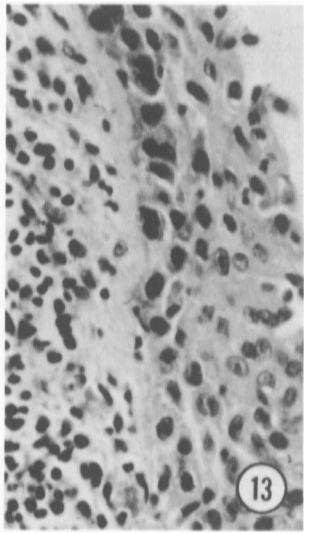
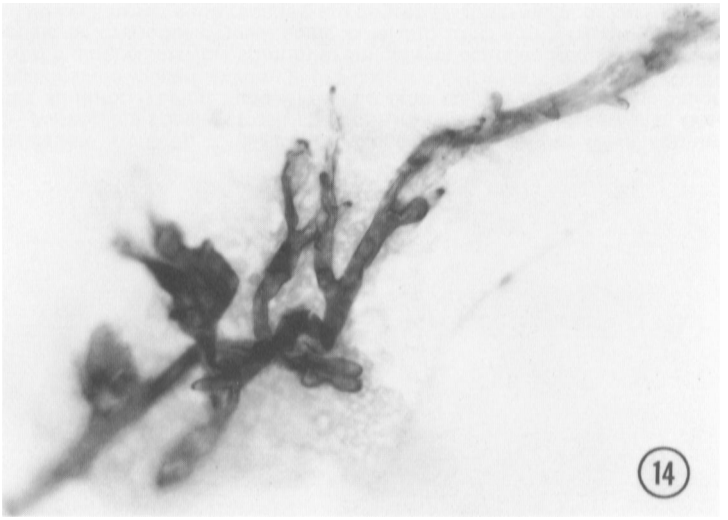
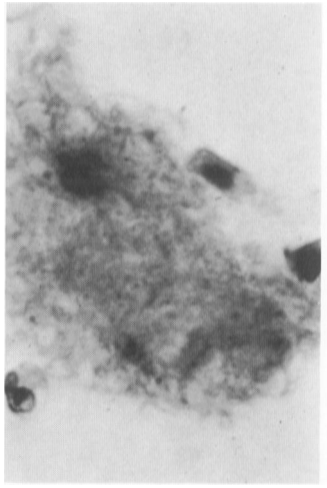


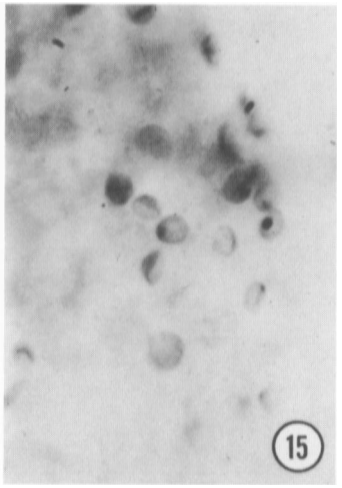
Figure 9—*Cryptococcus neoformans;* fresh sputum (Papanicolaou stain, original magnification × 1000). **Figure 10**—*Coccidioides immitis;* fresh sputum (Papanicolaou stain, original magnification × 400). **Figure 11 (left and middle panels)**—Aspergillus species; fresh sputum. Branching hyphal fragments. (Papanicolaou stain, original magnification × 400) **(right panel)**—Aspergillus species; fresh sputum. Fruiting head. (Papanicolaou stain, original magnification × 400). **Figure 12**—Squamous metaplasia and dysplasia, aspergilloma of lung; Saccomanno-treated sputum. In this case the patient presented with cough, minimal fever, and a lung mass which on x-ray examination suggested a carcinoma of the lung. Numerous sputum examinations contained both single cells and clusters of cells, as illustrated. The cells were consistently small, round orangeophilic or cyanophilic cells, some pale and others with hyperchromatic irregular nuclei. Some nuclei had small nucleoli, as in the cell in the lower left panel. The cells never quite reached the degree of abnormality of keratinizing squamous cell carcinoma. The lung mass with the associated lobe was resected. There was a cavity filled with aspergillus hyphae and squamous metaplasia and severe dysplasia of the adjacent bronchus. (Papanicolaou stain, original magnification × 400)











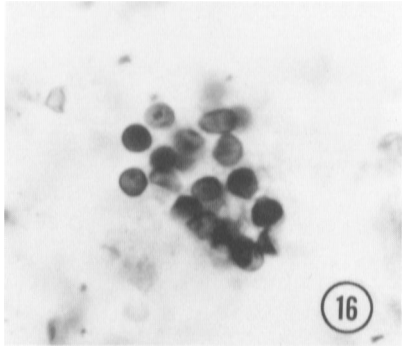
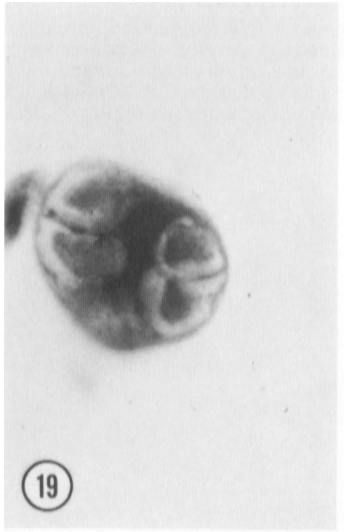
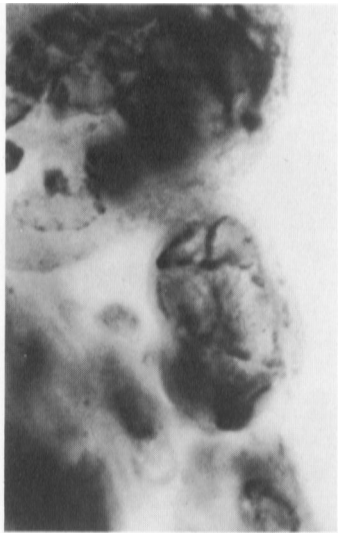
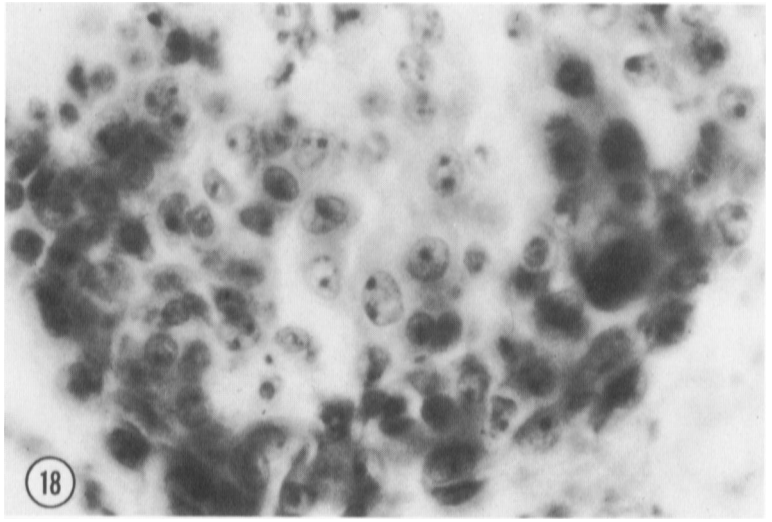


Figure 13 (left panel)—Aspergillus species present in cavitary lesions of lung diagnosed as aspergilloma. (H&E paraffin section, original magnification × 400) **(right panel)**—Wall of aspergilloma. Note the atypical epithelium. (H&E paraffin section, original magnification × 400). **Figure 14**—Nonseptate, branching hyphae seen in pulmonary phycomycosis; bronchial washing (Methenamine silver, original magnification × 400). **Figure 15 (left panel)**—*Pneumocystis carinii;* fresh postbronchoscopy sputum. (Papanicolaou stain, original magnification × 1000) **(right panel)** The area depicted in the left panel has been restained with methenamine silver. (Original magnification × 1000) **Figure 16**—*Pneumocystis carinii;* pulmonary lavage. Experimental pulmonary infection in mouse. (Methenamine silver stain, original magnification × 1000)







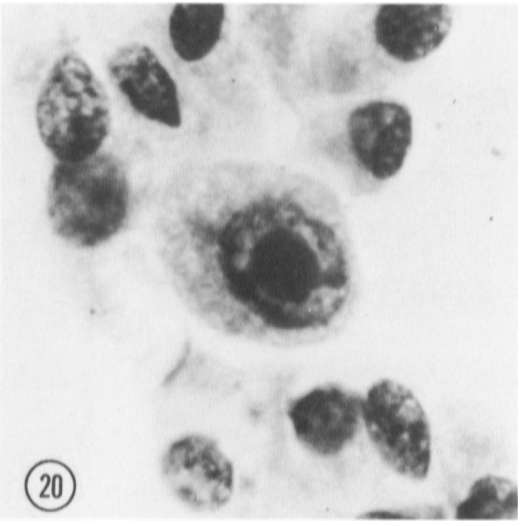


Figure 17—*Strongyloides stercoralis;* fresh sputum (Methenamine silver stain, original magnification × 400). **Figure 18**—Nonspecific reactivity of bronchial epithelium in presence of viral pneumonia; fresh sputum (Papanicolaou stain, original magnification × 400). **Figure 19**—Herpes simplex; fresh sputum (Papanicolaou stain, original magnification × 1000). **Figure 20**—Cytomegalic inclusion disease; bronchial brushing (Papanicolaou stain, original magnification × 1000).

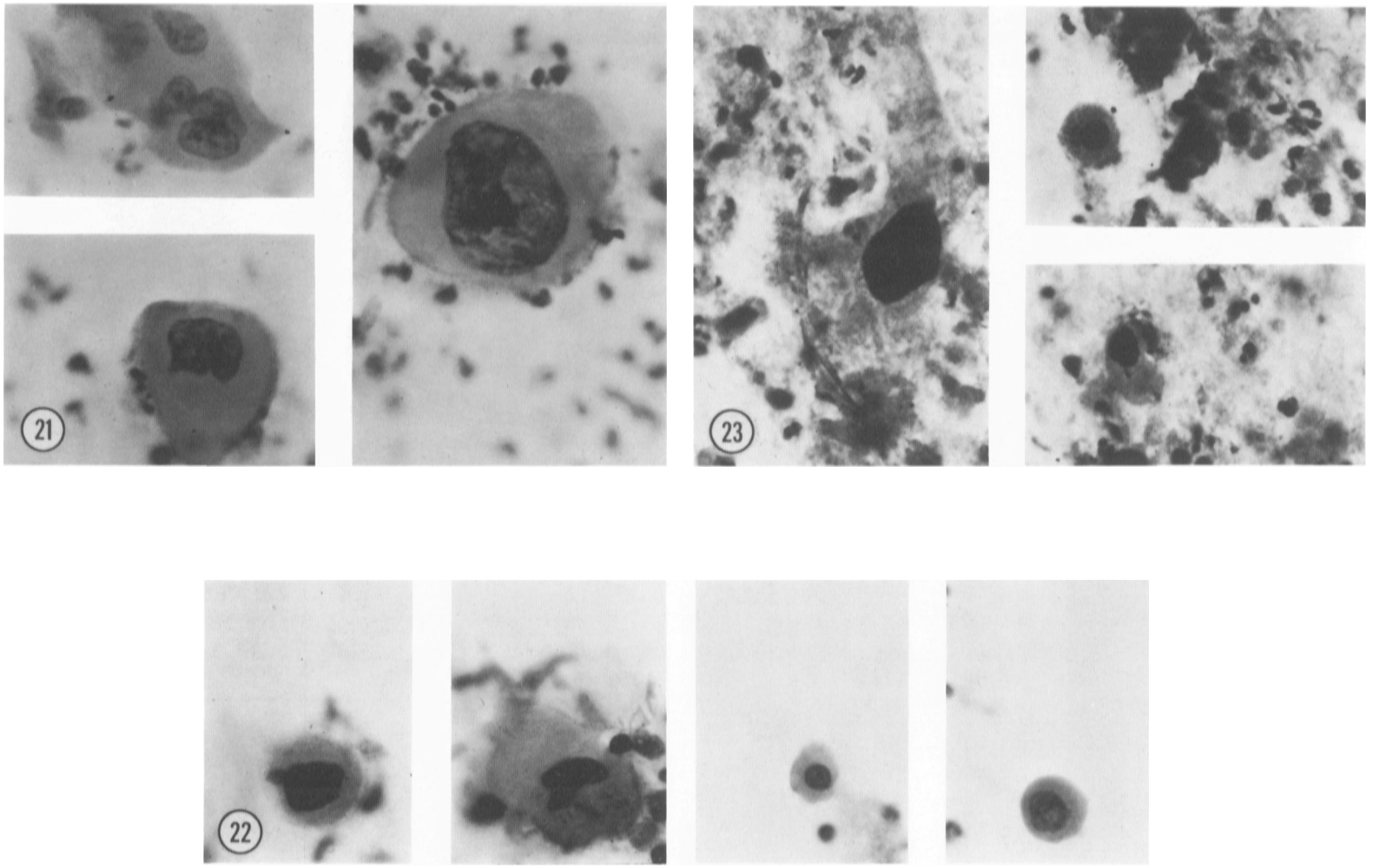


Figure 21—Keratinizing squamous cell carcinoma; Saccomanno sputum (Papanicolaou stain, original magnification × 400) **Figure 22**—Dysplastic squamous cells from a patient with squamous cell carcinoma of the lung **(left panels)** compared with dysplastic squamous cells from a patient with an inflammatory process **(right panels).** Note differences in preservation of nuclear chromatin. Saccommano sputum (Papanicolaou stain, original magnification × 400) **Figure 23**—Keratinizing squamous cell carcinoma with a cavity; Saccomanno sputum (Papanicolaou stain, original magnification × 400).

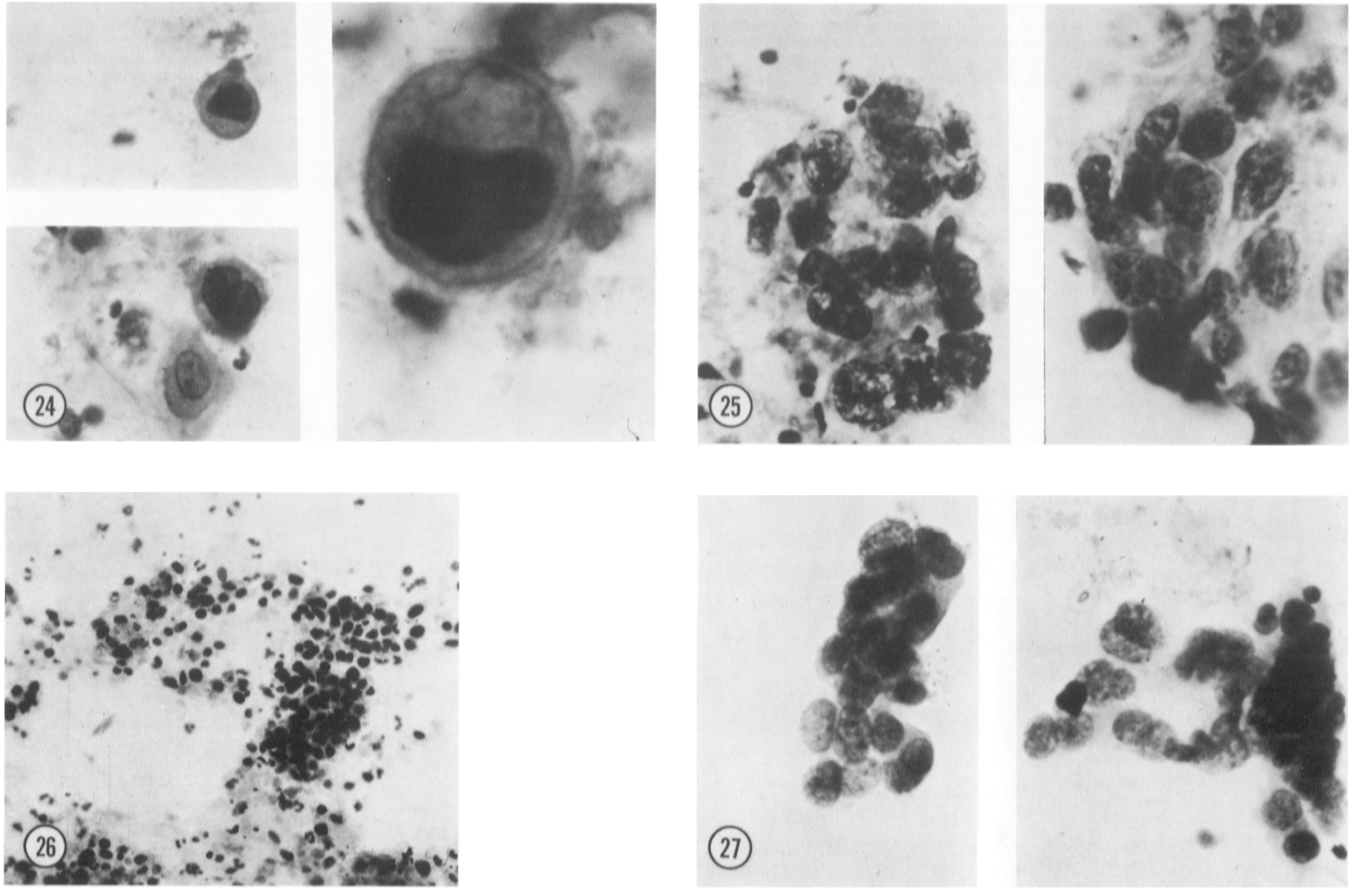
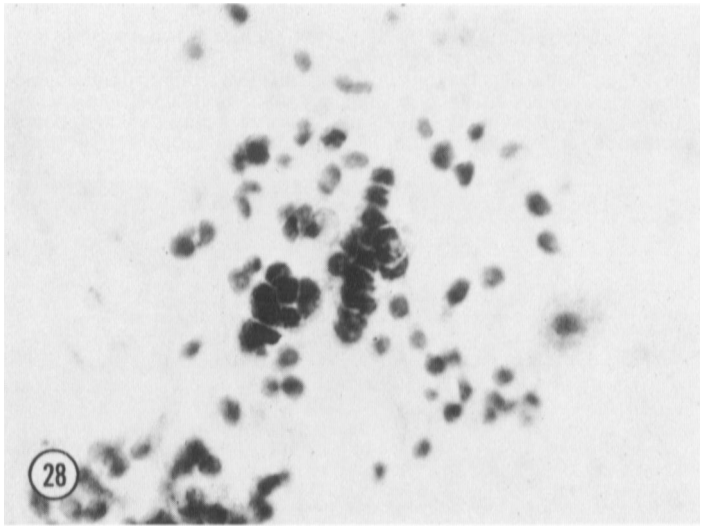
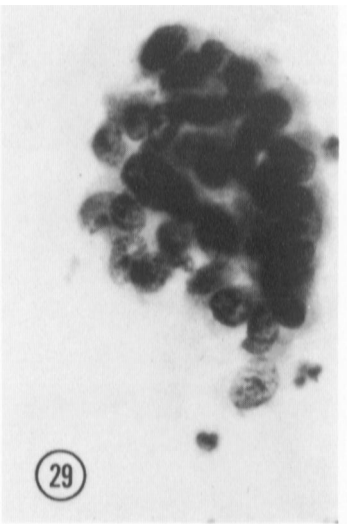
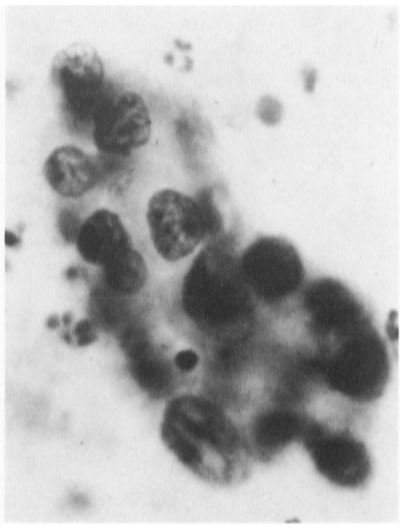
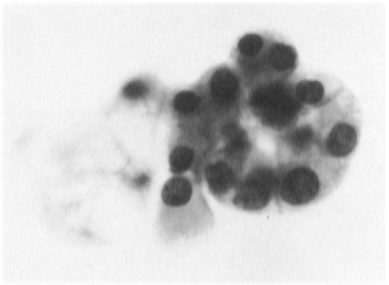


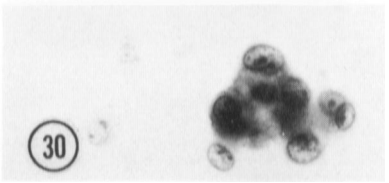
Figure 24—Cells from nonkeratinizing (poorly differentiated) squamous cell carcinoma illustrating double cell border that identifies them as of squamous type; Saccomanno sputum (Papanicolaou stain, original magnification, left panels, × 400; right panel, × 1000). **Figure 25**—Large cell undifferentiated carcinoma; bronchial brushing (Papanicolaou stain, original magnification × 200). **Figure 26**—Degenerated cells from large cell undifferentiated carcinoma in sputum; Saccomanno sputum. Note loose cluster with small irregular cells suggesting the diagnosis of small cell undifferentiated carcinoma. (Papanicolaou stain, original magnification × 400) **Figure 27**—Small cell undifferentiated carcinoma; bronchial brushing. Note prominent nuclear molding and size of the tumor cells in this type of specimen. (Papanicolaou stain, original magnification × 400).

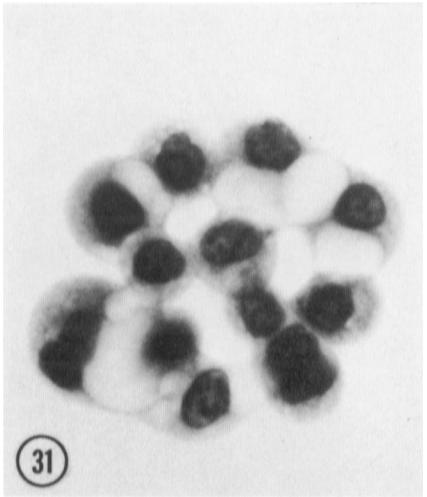


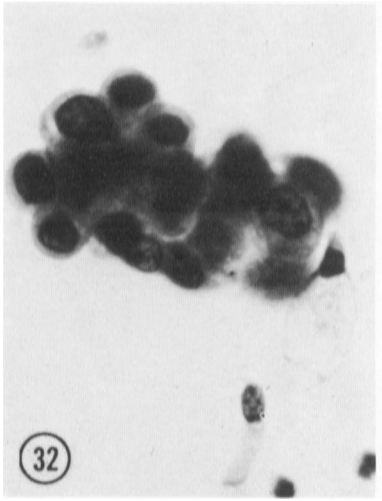












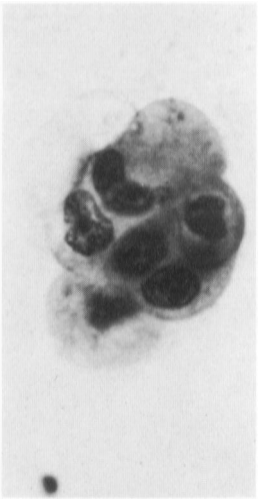


Figure 28—Small cell undifferentiated carcinoma; fresh sputum. Although the nuclear detail has been obliterated by karyopyknosis, the intense intercellular molding establishes the diagnosis. (Papanicolaou stain, original magnification × 100). **Figure 29**—Bronchioloalveolar cell carcinoma; Saccomanno sputum (Papanicolaou stain, original magnification × 400). **Figure 30**—Bronchioloalveolar cell carcinoma in cytology sample of fresh sputum **(top panel)** compared with same type of carcinoma in Saccomanno sputum **(bottom panel)**. Note sharply bordered vacuoles in the tumor cells in the fresh sputum preparation. See also Figure 31. (Papanicolaou stain, original magnification, × 400) **Figure 31**—Bronchioloalveolar cell carcinoma; fresh sputum (Papanicolaou stain, original magnification × 1000). **Figure 32**—Bronchogenic papillary adenocarcinoma. Saccomanno preparation of bronchial washings. Note clear to finely vacuolated cytoplasm of tumor cells. (Papanicolaou stain, original magnification ×1000)

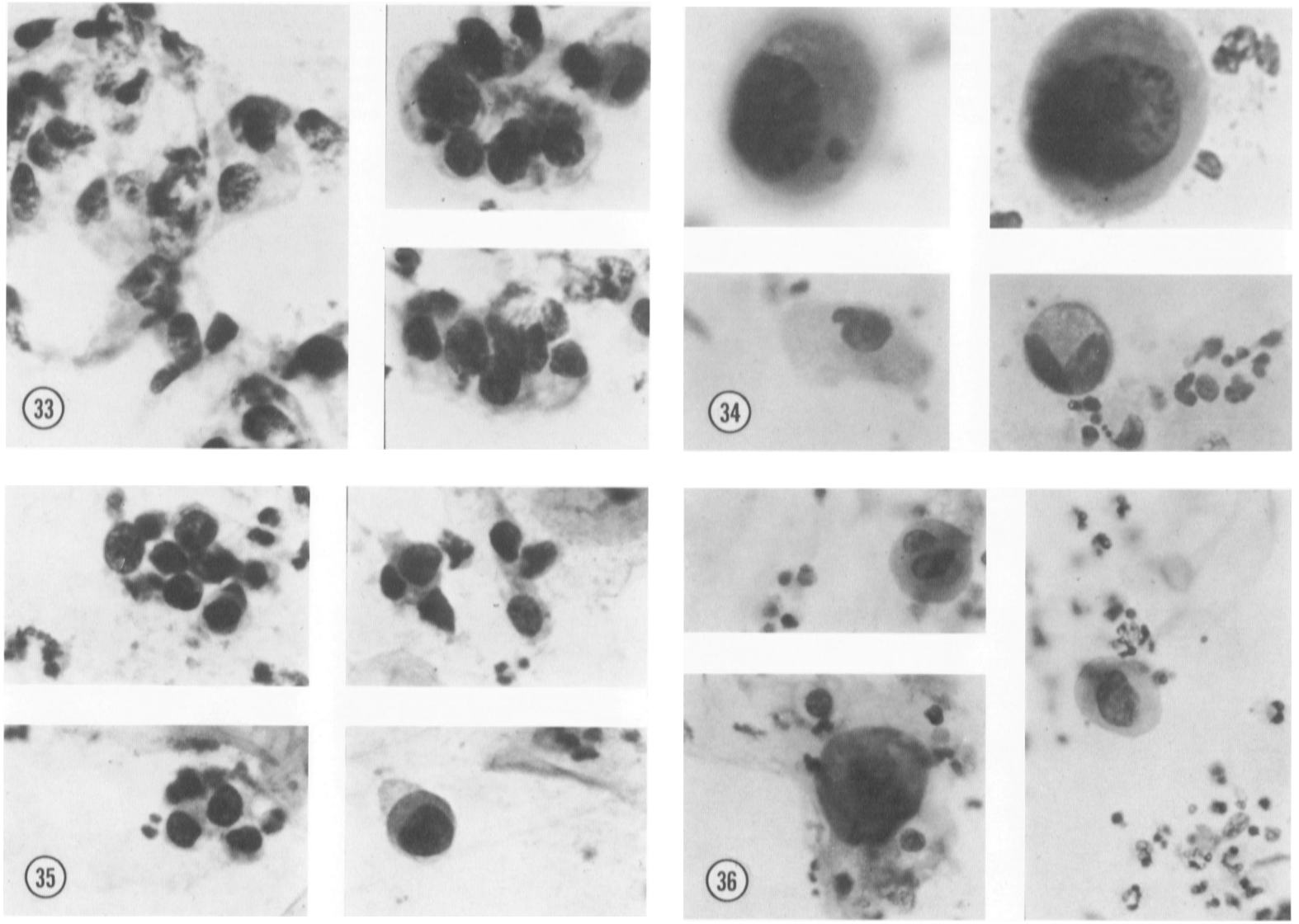


Figure 33—Bronchogenic acinar adenocarcinoma, poorly differentiated; bronchial washings (Papanicolaou stain, original magnification × 400) **Figure 34**—Giant cell carcinoma; Saccomanno sputum (Papanicolaou stain, Original magnification, upper panels, × 1000; lower panels, × 400). **Figure 35**—Mucoepidermoid carcinoma; Saccomanno sputum. Note clustering of cells as predominant feature with rare cells having irregular shape and dense cytoplasmic border indicating squamous component of the tumor. (Papanicolaou stain, original magnification × 400). **Figure 36**—Scattered dysplastic and pleomorphic keratinized cells from patient with occult and clinically early lung carcinoma; Saccomanno sputum (Papanicolaou stain, original magnification × 400).

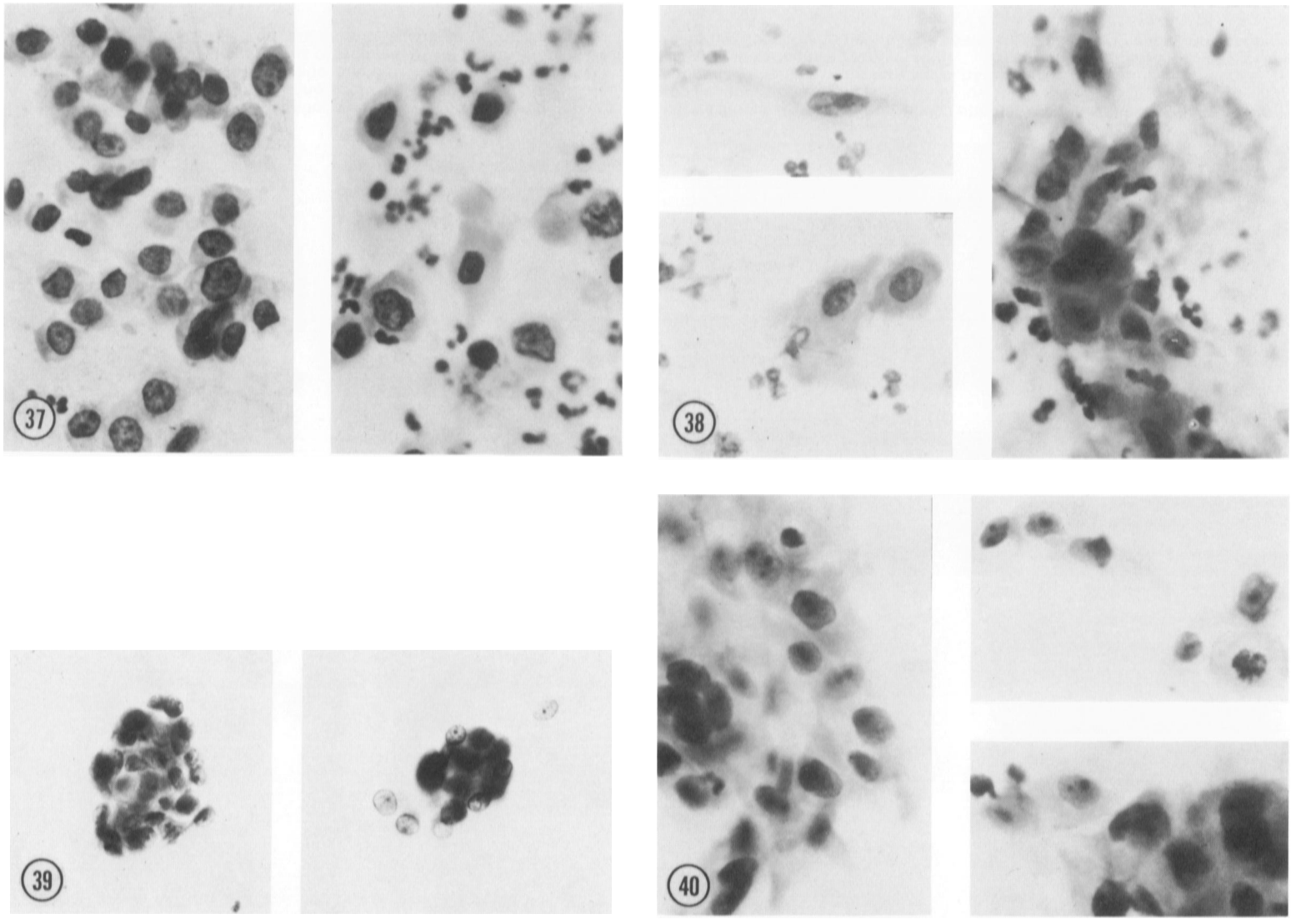


Figure 37—Malignant parabasal type squamous cells with few dysplastic cells from a patient with *in situ* and microinvasive squamous cell carcinoma of the lung; Saccomanno sputum (Papanicolaou stain, original magnification ×400). **Figure 38**—Metaplastic and dysplastic but reactive squamous cells; tracheal aspiration. Tracheostomy patients frequently have these cells which may be very atypical. (Papanicolaou stain, original magnification × 400) **Figure 39**—Sheets of reactive alveolar pneumocytes from a patient with adenovirus infection; bronchial washings. Note very prominent nucleoli within extremely pale and uniform background of nuclear chromatin. Nuclear/cytoplasmic ratio is not significantly altered. (Papanicolaou stain, original magnification × 400) **Figure 40**—Cellular changes from a patient with pulmonary infarct **(left panel)** compared to tumor cells from a patient with bronchioloalveolar cell carcinoma **(right panels)**; bronchial brushing. Note differences in spatial arrangement of the cells, particularly the absence of significant molding in the cell cluster from the carcinoma. (Papanicolaou stain, original magnification × 1000)