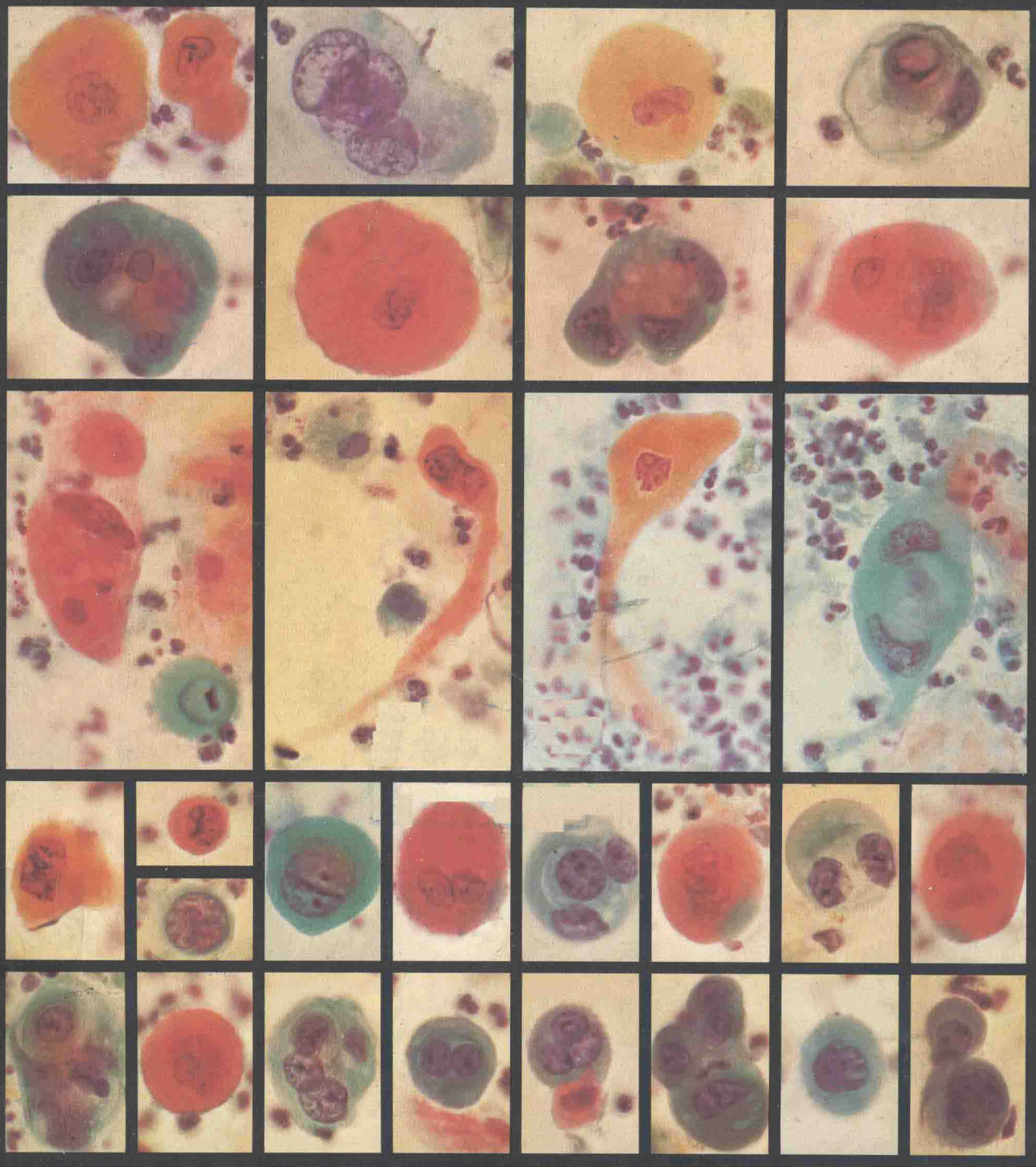
Diagnostic Pulmonary Cytology

GENO SACCOMANNO



AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

DIAGNOSTIC PULMONARY CYTOLOGY

Geno Saccomanno, PhD, MD
Pathologist
St. Mary's Hospital and Medical Center
Veterans Administration Hospital
Grand Junction, Colorado

Educational Products Division
American Society of Clinical Pathologists
Chicago

Library of Congress Cataloging in Publication Data
Saccomanno, Geno, 1915–
Diagnostic pulmonary cytology.
Bibliography:
Includes index.
1. Lungs—Cancer—Diagnosis—Atlases. 2. Diagnosis, Cytologic—Atlases. 3. Exfoliative cytology—Atlases. 4. Sputum—Examination—Atlases. I. Title. [DNLM: 1. Cytodiagnosis—Atlases. 2. Lung neoplasms—Diagnosis—Atlases. 3. Lung—Cytology—Atlases. WF17 S119d]
RC280.L8S23 616.9'94'24 78-8285
ISBN 0-89189-050-5

DIAGNOSTIC PULMONARY CYTOLOGY

To my wife, Virginia, for her patience, love, and understanding.

PREFACE

The primary objective of this text is to aid the cytotechnology student, the cytotechnologist, and the cytopathologist in the diagnosis of cancer of the lung. The cellular elements seen on slides prepared from sputum samples and pleural aspirates are shown by color photography. In addition to the cytological photographs, histological sections of most of the tumors are presented. The purpose of this presentation is to establish clearly the similarities and differences between the results of these two methods of studying pulmonary tumors. Often, the histology reveals the most common cell forming the tumor but this may or may not be the same as the cell most commonly identified on the smear.

All cells found in sputum—normal, inflammatory, premalignant, and malignant—have been indexed and catalogued so that the student can readily compare cells discussed in the text with those seen under the microscope. The details of collection, preparation, and staining methods are presented as lucidly as possible, and the importance of each of these phases is stressed.

The first eight plates show cells that represent the response of the lung both to acute and chronic inflammation, with discussion of the function of these cells and the frequency with which they are seen. Specific cell patterns in some diseases are demonstrated to assist in the identification of all cells present on the smear prepared from sputum.

Plates 9–15 are devoted to the histology and cytology of squamous cell metaplasia. Frequently, these cells are misdiagnosed as neoplasia and are poorly understood with regard to cause and significance; therefore, they are presented and classified on the basis of varying degrees of atypia.

Plates 16–44 demonstrate the histology and cytology of the most common tumors of the lung. Care has been taken to match the color of Papanicolaou or other good stains so that the same cellular features can be seen under the microscope. The student can readily find all neoplastic cells on the plates for comparison. An attempt has been made on most plates to present a wide range of cell patterns. These are discussed in detail.

Plates 45 and 46 show two interesting cases of mesothelioma with discussion of their behavior. Plates 47–65 demonstrate metastatic sputum cells and histological sections of the tumor exfoliating these cells. As a consequence of the advent of chemotherapy, advanced carcinogenesis is receiving more and more attention. In some cases, sputum cytology serves as an adequate monitor of chemotherapeutic response. This method of monitoring may prove even more effective as new anticarcinogenic drugs are found.

This work represents an effort to facilitate visual comparison of cells shown in the plates with those seen through the microscope on well-prepared, well-stained sputum slides. The purpose of this book, then, is to further early diagnosis, resulting in better patient care and increased patient survival.

ACKNOWLEDGMENTS

This text is the product of many dedicated persons interested in the constant improvement of care of the cancer patient. To those who contributed to this task, I am very grateful. Mr Jerry Scott, CT (ASCP), photographer, contributed

to the selection of cases from a cytological viewpoint, painstakingly photographed most of them and arranged the plates. His meticulous attention to detail speaks for itself. Mrs Lola Brennan, BS, CT (ASCP), Chief Cytotechnologist, St Mary's Hospital and Medical Center, assisted in selecting cases, researching materials, and reading the manuscript. Obviously, all of the students and cytotechnologists in St Mary's Department of Cytopathology contributed to this effort. The Department of Energy (Contract No. EY-76-C-02-1826) and the US Public Health Service and their representatives, particularly Joseph Goldstein, MD, Walter Weyzen, MD (Present Medical Director, Department of Energy, Washington, DC), and Victor Archer, MD deserve thanks for help in gaining financial support and for their constant encouragement. I acknowledge Oscar Auerbach, MD for his help and contribution of specimens from the Uranium Miner Lung Study. The staff members of St Mary's Department of Pathology, Drs R.P. Saunders, M.G. Klein, and J. Steinbrecher, are to be thanked for their encouragement and patience. The administrators, the physicians, and the patients both at St Mary's Hospital and at the Veterans Administration Hospital in Grand Junction, Colorado deserve thanks for their contributions. The editorial staff at the American Society of Clinical Pathologists was helpful and diligent in the preparation of the manuscript for final printing. And, finally, but not least, Mrs Willie L. Turner, secretary, deserves special thanks for her contribution in proofreading and typing.

TABLE OF CONTENTS

INTRODUCTION

PULMONARY CYTOLOGY

Cancer of the lung is the most common malignant neoplasm among the American male population[1] and is a rapidly increasing malignant lesion in the female population as well. Historically, cancer of the lung was an almost unheard of disease in the 18th century and a rarity in the 19th century.[2,3] The rarity of these tumors and the absence of X-rays and tissue sections, which were not available until the early part of the 19th century, made the differentiation between inflammatory diseases and neoplasia of the lung very difficult if not impossible. Virchow[4] was one of the first scientists to utilize cellular identification of tumors by microscopy in the diagnosis of lung lesions.

The first microscopic description of cancer of the lung was made by Quain in 1857.[5] The metastases from these lesions were first reported by Greene[6] in 1843. Bronchoscopy with direct visualization of the tracheobronchial tree, made possible for the first time by Killian,[7] completed the triad of histology, X-ray, and visual observation of the main bronchi, which aided in differentiating neoplasia from inflammatory diseases. At that time, tuberculosis was probably the most common chronic lung disease that had to be differentiated from lung neoplasia. Today, of course, the reverse is the case. Tumor is common, and tuberculosis is a rare lesion of the lung, at least in this country. With increased cigarette smoking and exposure to other respiratory carcinogens, a neoplasm of the lung rare in the previous century has become the most common in man today.

As the incidence of cancer of the lung increased, radiological diagnosis improved. Anesthesia and surgical treatment developed rapidly; nonetheless, the five-year survival period stabilized at only 5%, much less than expected. Even though our knowledge about these tumors increased, the response remained poor. It then became apparent that when lesions were large enough to be seen radiologically they were, in reality, in an advanced state of development and, all too frequently, had already spread to regional nodes and other areas. Means of identifying the incipient stages of tumor development became possible with the advent of sputum cytology.[8]

The art of sputum cytological diagnosis was not available during the first half of the 20th century. Papanicolaou, the father of modern cytology, presented his first case of the cytological diagnosis of lung cancer in 1945.[9] Although occasional cytological diagnoses had been reported previously, Papanicolaou was the first to use cytology for identification of these lesions, which initiated the technique as a worthwhile procedure.

With better techniques of sputum collection, preservation, and preparation, the diagnosis of lung cancer improved and sputum cytology became common in the modern hospital laboratory. Moreover, pulmonary cytology has become an accepted method of screening in the identification of the effects of single and multiple carcinogens in high-risk populations. Metastatic malignancies to the lung are also readily identified by sputum cytology, which will prove to be valuable in monitoring advanced disease, particularly in assessing the effectiveness of chemotherapy.

Fiberoptic bronchoscopy has contributed materially to the localization of lung tumors. This method enables the diagnosis of very small, as well as large, lesions. With continued research, it should allow localization of early in situ cancer of the lung and improve the five-year survival period.[10]

All of these methods of diagnosis and treatment of lung cancer are, at present, poorly integrated and probably not yielding much improvement in the five-year survival period. However, all of these techniques are being improved rapidly and will contribute tremendously in the near future. Many novel ideas in immunology, fluorescence,[11] fiberoptics, and cytotechnology show much promise in this field.

Keratinizing epidermoid cancer of the lung, the most common lung carcinoma, is readily diagnosed in sputum smears because of bizarre cytological patterns and an abundance of tumor cells. Many of the other cancers of the lung, however, are difficult to diagnose because of subtle cellular patterns, fewer cells, and rarity as lung lesions.

It is the object of this text to present cases and discussions which, it is hoped, will provide a ready reference to the student in cytotechnology training, the cytotechnologist, and cytopathologist. The squamous and adenocarcinomas of the lung vary markedly, histologically and cytologically; therefore, where justified, several tumors of each type are presented to familiarize the student with these variants. Using histological and cytological photographs, this work presents premalignant and malignant tumor cells found in the sputum and originating in primary and metastatic tumors of the lung.

REFERENCES

1. Silverberg E: Cancer statistics, 1977. CA 27:26–41, 1977
2. Morgagni JB: De sedibus et causi morborum. Lavanii Typogr. Acad., 1761, Lib. II, ep. 22 (quoted by Pepper[3])
3. Pepper W: Cases of cancer of the lungs and mediastinum. Trans Stud Coll Physicians Phila 1:96–110, 1850
4. Virchow R: Cellular Pathology. London: J. Churchill, 1860, p 479
5. Quain F: Encephaloid tumor involving the heart and lungs. Br Med J 44:902, 1857
6. Greene S: Encephaloid tumours in the brain and lungs. Dublin J Med Sci 24:282–283, 1843
7. Killian G: Uber Directe Bronchoskopie. Munch Med Wochenschr 45:844–874, 1898
8. Saccomanno G, et al: Cancer of the lung; the cytology of sputum prior to the development of carcinoma. Acta Cytol 9:413–423, 1965
9. Papanicolaou GN: Diagnostic value of exfoliated cells from cancerous tissues. JAMA 131:372–378, 1946
10. Woolner LB, et al: In-situ and early invasive bronchogenic carcinoma: report of 28 cases with post-operative survival data. J Thorac Cardiovasc Surg 60:275, 1970
11. Profio AE, Doiron DR: A feasibility study of the use of fluorescence bronchoscopy for localization of small lung tumors. Phys Med Biol 22:949–957, 1977

MATERIALS AND METHODS

The field of pulmonary cytology embraces many phases of technology which must be integrated to yield ideal results. The quality and quantity of the specimen are dependent on collection, preservation, preparation, staining, and screening, which are some of the phases of sputum cytology that must be handled carefully, or the end product will be inadequate for a correct diagnosis. Each step is a link in a chain, which is only as strong as its weakest link. Obviously, if the sample collected is inadequate, not primarily of lung origin, the correct diagnosis will not be possible even after efficient processing. If the fixative is inadequate, although the sample may be directly from the tumor, and the processing, staining, and reading are done very efficiently, the cells will be degenerated, making an accurate diagnosis impossible. Every step in the process must be perfect if a definitive diagnosis is to be made. A detailed description[1] of the collection, preparation, staining, and reading of material is given in the next section.

In the assessment of sputum slide material, the student is encouraged to identify all cells seen in the smear. Recognition of the presence or absence of tumor cells is the primary objective of the proper reading of a sputum smear; however, recognition of all cells present will elicit other worthwhile information. The amount of inflammation present allows assessment of the presence and degree of chronic obstructive pulmonary disease.[2] The presence of eosinophils admixed in the exudate may reveal an allergic reaction or Löffler's syndrome(see Plate 8). The identification of the various degrees of squamous metaplasia gives some assessment of premalignant cellular changes in the bronchial tree and indirectly suggests the degree of carcinogenic exposure or sensitivity of the patient to a particular carcinogen.

Identification of all cellular elements is encouraged throughout this text and played an important role in the selection of material for photography. The presence of macrophages and tall columnar cells indicates that the specimen is of lung origin; therefore one must be constantly aware of these cellular elements to ensure that the specimen is indeed of lung origin and adequate. Unsatisfactory specimens always leave a void—the patient may have a malignancy, but the diagnosis cannot be made. It is important to evaluate a specimen properly, and if it is inadequate in some way, that should be acknowledged. It is misleading to call a specimen satisfactory when it is not, because this gives the physician a false sense of security. Evaluation of the various qualities of each specimen are discussed.

The most common lesions of the lungs, larynx, and pleura are presented in this text and shown histologically and cytologically. Examination of the tissue sections frequently reveals characteristics which may shed light on the cytology. Variations of cell size and arrangement, preservation, and necrosis of cells are seen most vividly in tissues and account for many of the variations noted cytologically. For example, when one examines a section of malignant tumor, one notes that the cell size varies markedly, but it is still obvious that all of the cells in the tumor are components of it. While examining a smear on which cells are scattered, one tends to be impressed with the larger cells, frequently discounting smaller ones. The same consideration applies to the nuclear/

cytoplasmic (N/C) ratio. Cells with increased N/C ratio attract attention readily, while those with a normal N/C ratio, even though having all the other features of malignancy, are not nearly as impressive and may be overlooked.

Of course, the cytology of a lesion is most important because it is the only element available for establishing the diagnosis. Most of the frames in this text are of cytology, and an attempt is made to show as wide a variety of cells as possible. Particular attention, wherever possible, is paid to cell size. The cells were not accurately measured but were estimated by comparison to polymorphonuclear leukocytes, which measure about 8–9 μ in diameter. Cell size is a very important tool, since some cells are similar in all observable characteristics such as cytoplasm, staining, and nuclear features, except size. Note some of the cells in the undifferentiated epidermoid tumors in Plates 24, 25, and 27 and compare these with the tumor cells seen in some of the oat cell carcinomas in Plates 41 and 42. Size of cells is a very important distinguishing feature in the identification of these tumors.

The cytological photographs are intended to simulate, as closely as possible, the cells seen microscopically in sputum under a 15× ocular in both low and high power. The commentary on the opposite page gives age and smoking history when this is important. Some comments about X-rays are also included when pertinent to the discussion as well as remarks about salient features of the histology. In the discussion of the cells, an attempt is made to orient the reader to size and staining features, with emphasis on the features that contribute to the diagnosis of a benign cell pattern or malignant tumor. Particular attention is paid to the features of the nucleus, because frequently it is well preserved while the cytoplasm is degenerate.

Most of the specimens selected for presentation in this text were collected from inpatients and outpatients at St Mary's and Veterans Administration Hospitals. The histological material is from lung biopsies, resected lesions of the lung, or from autopsies. The remaining specimens were collected in the course of the uranium miner lung study on the Colorado Plateau. This study has been in progress for 20 years and involves the collection of sputum samples and tissue from miners who developed cancer of the lung. Many of these patients had provided sputum samples for several years prior to the development of cancer. These studies have proven worthwhile, particularly in developing insight on how tumors develop in the premalignant as well as malignant stages. It is of interest to mention here that cancer of the lung in uranium miners is no different in its development from tumors that develop in cigarette smokers, except that the addition of the second carcinogen (radiation) results in a slight increase in the incidence of small cell tumors of the lung.[3, 4, 5]

Whenever possible, the tissue sections are from the same tumor that released the cells collected in the sputum. When this was not possible, matching of tumor and cell types was made to show similar patterns. All of the tissue sections were stained with Mayer's hematoxylin and eosin (H&E). All sputum samples were prepared with the Saccomanno technique[6] and stained with Papanicolaou stain. All photographs were taken with a Zeiss photomicroscope II on Kodacolor-X and Kodacolor II film and printed on Kodak paper. The photos were taken with Planapo 40 oil immersion objective, resulting in a magnification of ×600. A 6.3 × Neofluar objective was used for the low-power ×100 magnification and a Planapo objective was used for the medium magnification of ×400. As mentioned elsewhere, all cells can readily be measured by comparison with

the size of a polymorphonuclear leukocyte or lymphocyte, which average between 8–10 μ.

REFERENCES

1. Saccomanno G: Sputum cytology: collection, fixation, and concentration of sputum, bronchial aspirates, and bronchial brushings. ASCP Technical Improvement Service, No. 27, 1976
2. Mittman C, Stevens D, Teplitz R: Cellular elements in sputum as an index of obstructive lung disease. (Unpublished)
3. Auerbach O, et al: Changes in the bronchial epithelium in relation to smoking and cancer of the lung. N Engl J Med 256:97–104, 1957
4. Saccomanno G, et al: Cancer of the lung; the cytology of sputum prior to the development of carcinoma. Acta Cytol 9:413–423, 1965
5. Saccomanno G, et al: Development of carcinoma of the lung as reflected in exfoliated cells. Cancer 33:256–270, 1974
6. Saccomanno G, et al: Concentration of carcinoma or atypical cells in sputum. Acta Cytol 7:305–310, 1963

SPUTUM CYTOLOGY: COLLECTION, FIXATION, AND CONCENTRATION OF SPUTUM, BRONCHIAL ASPIRATES, AND BRONCHIAL BRUSHINGS

CLINICAL RATIONALE

Normally, the epithelium of the tracheobronchial tree has a total cellular turnover of 30 days.[1] This turnover results in an abundance of epithelial cellular discharge in the lumen of the bronchial tree. Many of these cells degenerate, fragment, and are not found in the expectorated sputum. Many, however, are found in the sputum in various stages of preservation. Some appear normal, others show fragmentation and swelling, and many may simulate tumor cells. Inflammatory cells are usually abundant, particularly in bronchitis, and may also be well preserved and/or degenerated. Effects of inflammation and respiratory carcinogens also cause cellular changes in the respiratory epithelium.

The lining cells of the tracheobronchial tree respond in a variety of ways to inflammation and exposure to respiratory carcinogens throughout long periods. This epithelium undergoes hyperplasia in the initial periods of insult, but if this is prolonged, be it inflammation or carcinogen, areas of squamous cell metaplasia develop. Initially, these areas of squamous cell metaplasia are usually small. They are most frequently noted on the bronchial spurs, extending for very short distances and are either abruptly or gradually joined by normal, tall columnar cells. With continued exposure, these areas of metaplasia increase in size and gradually become more and more cytologically atypical. In a few cases,[2] they may eventually become malignant.

During this process of tumorigenesis, the epithelium, normal and abnormal, continues to shed cells into the sputum and is subject to cytological examination. A sputum sample that is of lung origin will therefore show a variety of cells representative of what is actually being shed by the lung lining at that time. If metaplasia is present, metaplastic cells are found in the sputum. If tumor is present, tumor cells will be found in the sputum, so that a sputum sample is a good index of what is being shed from the whole bronchial tree. Tumors, however, develop in isolated areas, and the problem then becomes one of localization of the lesion. Roentgenograms of the chest are helpful if the lesion is large enough to be seen; however, this is a late finding. Localization of lesions smaller than those visible on X-ray film is difficult, necessitating direct visualization with rigid or fiberoptic bronchoscopy. Confirmation with either tissue biopsy or smear specimens from the suspicious area can then be made. Therefore, a variety of specimens must be examined, depending on such factors as the presence or absence of neoplasia and the size of the lesion. The specimens to be examined consist of the following: (1) sputum, to determine the degree of disease present; (2) bronchial washings, used in an attempt to localize the lesion to a

specific lung or lobe; (3) bronchial brushings, to assess abnormal-appearing areas; and (4) biopsy tissue, taken from areas suspected of having tumor. These various specimens and their manner of preparation will be described separately.

SPUTUM COLLECTION, PRESERVATION, AND PREPARATION OF SLIDES FOR CYTOLOGICAL STUDY

The object is to collect a satisfactory amount of sputum of pulmonary origin. The patient should not only be informed that it is essential that an adequate amount of sputum is necessary but should also be given some information on the physiology and anatomy of the respiratory system, with detailed instructions that the sputum needed must come from "deep in the lungs." One must explain that such materials as nasal aspirates and tongue scrapings, not being of pulmonary origin, are not what is desired. Sometimes this is a difficult point to put across, particularly if the patient is frightened or anxious about the diagnosis. The patient must be at ease with the person explaining the procedure, whether that is collection of a sputum sample the following morning or presently the use of an aerosol. It has been our experience that patients are frequently so worried that they hear little of the instructions and must be put at ease. Following the instructions, it is sometimes worthwhile to ask patients direct questions to confirm that they know what you are talking about. Once the patient knows what is desired, the specimen can be collected in two ways: at home on first arising from bed or by use of an aerosol. The collection of sputum at home will be discussed first.

Usually, patients with diagnostic lung problems are cigarette smokers and, after the discussion on the type of sputum sample desired, know exactly what is needed. They will readily admit that on arising from bed they cough up material from deep in the lungs. A rare person, although a cigarette smoker, will not be able to produce sufficient cough material for study, but 90% of all patients will yield a satisfactory specimen spontaneously on arising from a night's sleep. The remaining 10% of the patients who do not cough must be administered an aerosol.

EARLY MORNING SPECIMENS FOR PULMONARY CYTOLOGICAL EXAMINATION

The patient is given three wide-mouthed specimen bottles, each containing 50 ml of fixative, consisting of 48 ml of 50% ethyl alcohol diluted from 95% ethyl alcohol, to which has been added 1 ml of 50% polyethylene glycol (Carbowax 1540) (Union Carbide Corporation, New York, NY) and 1 ml of ethyl alcohol containing 3 mg of Rifampin (Dow Chemical Company, Midland, MI). It should be explained that this is not a potable alcohol and must be used for fixation only. Staining material, such as eosin or methylene blue, may be added to prevent drinking of the fixative. The patients are instructed to use one container each morning, not adding more than 15–20 ml or the equivalent of four or five tablespoons of sputum. They are instructed to rinse their mouths with water on arising. One of the bottles should be available, and, on coughing, the patient

should spit into the bottle, firmly tighten the lid, and shake vigorously for a few seconds. The procedure is repeated until the estimated amount of four to five tablespoons of sputum is added to the bottle. This is done for three consecutive mornings. These three specimens are returned to the laboratory for processing, where they are pooled into one.

The noncoughing patients are administered an aerosol (Fig. 1 on p 11), which may be accomplished in the following manner. Various aerosol instruments can be used. When available, we prefer the one by DeVilbiss (Toledo, OH); however, the Monaghan Ultrasonic Nebulizer (Pueblo, CO) can be used. The object of aerosolization is to introduce a significant amount of water into the lungs. Irritants or mucolytic agents can be added, but our experience with these has not yielded any improvement over tap water. It is most important again to explain to the patient the procedure and the expected result. After 20 to 30 minutes of aerosol inspiration, the patient should take at least four deep breaths, and on the fifth, forcefully attempt to cough. This procedure should be repeated with reasonable rest periods between attempts. Sometimes, if the patient cannot produce an adequate sample using an aerosol, he will have a productive cough within the next 24 hours. Therefore, the patient should be given a bottle containing fixative and instructions for collecting a sputum sample during this period of time. The sputum specimen should always be collected after the patient washes his or her mouth with water. The specimen is expectorated into a large-mouth bottle, about half full of 50% ethyl alcohol (95% ethyl alcohol diluted to 50%; do not use absolute), containing 1 ml of 50% polyethylene glycol (Carbowax 1540) and 1 ml of 50% ethyl alcohol containing 3 mg of Rifampin.

PROCESSING OF FIXED SPUTUM SAMPLES COLLECTED SPONTANEOUSLY OR WITH AN AEROSOL

It should be mentioned that the fixative of 50% ethyl alcohol plus Carbowax has been found to adequately sterilize the specimens in our laboratory; however, it is advised that we are adding 1 ml of 50% ethyl alcohol containing 3 mg of Rifampin to the fixative in each bottle prior to collection. On arrival of the specimen at the laboratory, another equal amount of Rifampin is added. The specimen is processed after 24 hours. If a negative pressure hood is available, the blending should be done in the hood. These are added precautions that should be taken, particularly if the specimens are from populations showing a high incidence of mycobacteria. In areas of high incidence of pathogenic fungi, antifungal amphotericin B should probably be added to the fixative before processing.

PROCEDURE

The specimen bottle lid is removed, and visual inspection of the sample is made to note its color and coarseness, or whether it is composed of large mucous blobs. This is important in assessing the blending time. If the material is pale and cloudy, the blending can be kept to a minimum; if large mucous blobs or thick masses of brown material are noted, then blending must be longer. Blending does not fragment cells but does create currents that, at 22,000 rpm,