

CYTOLOGIC DIAGNOSIS OF LUNG CANCER

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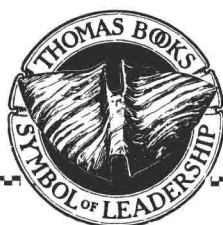
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DEDICATION

To Herbert F. Traut

BEFORE the turn of the century, Lataste demonstrated the cyclic changes of the vaginal epithelium of rodents. Numerous investigators corroborated this reaction to periodic fluctuations of sex hormones. In 1917, Stockard and Papanicolaou demonstrated that the cycle could be followed by a study of vaginal smears which obviated the necessity for sacrifice of the animal. This furnished the technical basis for the localization and identification of the ovarian hormones.

In 1938, George N. Papanicolaou joined Herbert F. Traut in a comparative study of the epithelial structures of the human genital tract. In this work they sought to correlate morphologic and functional characteristics of the entire female genital epithelial surfaces with the cellular content of the vaginal smear. Many pathologic conditions were found to interfere with normal morphology to an extent not anticipated

from previous studies in animals. While many isolated observations of exfoliated malignant cells had been made in previous years, these workers first demonstrated the feasibility of detecting cancer in a majority of cases by the demonstration of exfoliated malignant cells in the vaginal smear. This contribution was made possible by Dr. Traut's great experience as a clinical gynecologist and as a gynecological pathologist in combination with Dr. Papanicolaou's development of and experience with the smear technique.

The work of many investigators through the years has confirmed this pioneer clinical evaluation.

Dr. Traut's interest and enthusiasm have served as a stimulus and inspiration to many workers including the authors of this monograph. His experience has contributed an invaluable guidance to this project.

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FOREWORD

IN SPITE of great technological advances in thoracic surgery and the treatment of lung cancer, mortality from the disease remains high and the factor of delay in diagnosis has changed but negligibly in the past two decades. Obviously, the great need today is a method which will permit earlier diagnosis. It is to be hoped that the application in competent, experienced hands of the cytologic diagnostic technique in the study of sputum and bronchial washings will represent a signal advance in this regard. It is to date the most promising of all diagnostic techniques for those cases of lung cancer with minor symptoms or with peripheral lesions where bronchoscopy is of no avail and successful biopsy not possible. Tragically, much of our present diagnostic armamentarium is of little help during the time when early lung cancer is curable. Valuable time is lost due to limitations and inadequacies of many of the complex diagnostic procedures in current use, and frequently months may pass before sufficient "evidence" can be crystallized into a questionable or a positive diagnosis of neoplastic pulmonary disease.

The procedure of cytologic study of sputum and bronchial washings is of itself quite simple and relatively inexpensive. Interpretation, on the other hand, of cytologic smear preparations is often difficult, and requires an almost complete reorientation of criteria as compared to those employed in histologic diagnosis. Therefore, it demands careful, meticulous training of the cytologist upon whose experience and

mature judgment successful use of the technique will depend.

This work constitutes the first authoritative, original monograph devoted solely to cytologic diagnosis of lung cancer. Its appearance is, therefore, most timely. The authors have based their conclusions upon the study of 1,526 cases representing a wide scope of disease, neoplastic as well as non-neoplastic. This has given them a broad experience and familiarity with the many possible variations which might occur in cells from different types of pathologic lesions. Obviously, this field of research has not been completely explored. The findings to date, however, should be a stimulus to further expanded study not only in primary lung malignancies, leukemia, and metastatic cancer but other diseases.

The results set forth in this study emphasize that the sensitivity of the procedure varies directly with the demands on its reliability. That is, dismissal of equivocal cells results in a minimal number of false positives. No single criterion is sufficient. Multiple criteria must be used in order to indicate unqualified presence of malignancy.

Using the Papanicolaou stain the authors have described methods whereby sputum and bronchial washings might be best selected, prepared, fixed and stained. Through careful studies they have shown that malignant cells can be identified with a minimum of false positives and negatives. Accuracy of diagnosis varies directly with the experience of the cytolo-

gist thus accounting for many of the errors referable to false positives in earlier studies. In this study the series of cases has had a large proportion of control material from patients in whom the possibility of cancer was remote. Cytologic diagnoses were rendered by individuals having no prior knowledge of the clinical status of the patients. Extensive follow-up of patients with negative as well as positive cytologic diagnoses has been conducted over long periods of time in order to get a valid estimate of the number of patients whose cancer will or will not be detected by cytologic technique. In this manner it has been possible to evaluate the method referable to the

usual dangers of gross error.

This monograph which has been written in eleven chapters and illustrated by ten color plates, each of which possesses six in-sets, should prove extremely valuable to all investigators and clinicians interested in earlier diagnosis of lung cancer and subsequent reduction in its mortality. In the hands of the cytologist, phthisiologist, oncologist, pathologist, internist, cancer control directors and other interested workers, this monograph will be a stimulus for further work.

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CYTOLOGIC DIAGNOSIS
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CHAPTER I

INTRODUCTION

BRONCHOGENIC carcinoma is one of the most common cancers and apparently its incidence is increasing. Despite the advances in thoracic surgery, the prognosis of a patient with bronchogenic carcinoma remains poor. Pulmonary cancer presents difficult problems to the physician and, in most instances, he cannot attack these effectively since his diagnostic methods fail to reveal the lesion often and early enough.

INCIDENCE OF PULMONARY CANCER.—Carcinoma of the lung comprises about 1 per cent of all autopsies and about 10 per cent of all cancer found at autopsy. Dublin¹⁶ estimates that each year approximately 15,000 patients in the United States die of bronchogenic carcinoma. Graham et al.³⁵ estimate that bronchogenic carcinoma accounted for about 0.54 per cent of all cancers found by autopsy in 1895. The present figure of 10 per cent would seem to indicate a twenty-fold increase in the incidence since that date. Necropsy statistics throughout the world show a corresponding increase in the incidence. There can be little doubt that lung cancer is more frequently recognized than previously.

Many investigators have concluded that the incidence of bronchogenic carcinoma has increased absolutely as well as relatively.^{44, 80, 87} On the other hand, Fried²⁹ and others believe that the increase is more apparent than real, and that it is based on better methods of diagnosis, increased attention to the disease, more frequent hos-

pitalization of patients, more available medical advice, and greater longevity.

Although the incidence of bronchogenic carcinoma has apparently increased, that of other forms of cancer has remained relatively stable. At present bronchogenic carcinoma is next in frequency to carcinoma of the stomach and of the large intestine in males.^{3, 51, 81} Halpert has predicted that carcinoma of the lung will become the most common malignancy in males.³⁷

All autopsy series present comparable figures regarding age distribution of carcinoma of the lung. The disease occurs most frequently in the years between 40 and 70.^{28, 88} Farber and Tobias¹⁹ report that in their 200 autopsied cases of bronchogenic carcinoma 90 per cent occurred in the 40 to 80 age group. Nevertheless, this cancer may occur in younger people. Perrone and Levinson⁸¹ have found 13 reported cases in which the patients were less than 19 years of age.

All series indicate that carcinoma of the lung occurs more frequently among males than females.^{75, 92, 96} Ochsner et al.⁷⁴ analyzing 8,575 collected cases, found that 78.9 per cent were males, a sex ratio of about 4:1. The age distribution is about the same for men and women.¹⁹ Dorn¹⁵ has pointed out that from 1930 to 1940 the death rate from cancer of the lung increased about 80 per cent in males, whereas it increased only 20 per cent in females. In all forms of cancer there was a 10 per cent increase in males and 1 per cent increase

among females during the same period.

Some evidence suggests a higher incidence of bronchogenic carcinoma among whites than among negroes,^{3,74} but this evidence is by no means conclusive.⁶⁴ A study by Ochsner⁷⁴ indicates that the increased incidence of bronchogenic carcinoma in New Orleans occurred primarily in white patients and showed little or no change among colored patients.

STATUS OF THERAPY.—Remarkable progress has been made in the therapeutic techniques applicable to carcinoma of the lung. Graham and Singer³⁴ reported the first successful pneumonectomy in 1933. Since that time development of greater operative skill, increased knowledge of fluid balance, and availability of effective antibiotics, have all contributed to reduction of operative mortality to as low as 3.3–4 per cent in some series.^{1,50} Despite these advances the five year cure rate remains discouragingly low. Ochsner et al.⁷⁴ have been able to report a 5 year survival rate in only 8 per cent of all their cases of bronchogenic carcinoma.

At present, the only hope for cure of carcinoma of the lung lies in complete removal of all the cancerous tissue by means of surgery. Obviously, this hope can be fulfilled only if the cancer is detected before it has developed local extension and metastases to the regional lymph nodes or to distant parts of the body.

DIAGNOSTIC PROBLEMS.—It is clearly evident from numerous current reports that many failures in the therapy of bronchogenic carcinoma may be partly attributable to late and inadequate diagnoses. Diagnostic methods have failed to keep pace with the progress of therapeutic skills:

Overholt and Schmidt, for instance, have analyzed the case histories of 604 patients seen between 1932 and 1948 to determine whether or not there had been any change in the time required to make a diagnosis of carcinoma of the lung.⁷⁶ They found that between 1932 and 1942, there was an average of $11\frac{3}{4}$ months between the onset of symptoms and the diagnosis; and that between 1942 and 1948 the interval had only been reduced to 10 months. Bearing in mind this time required to make the diagnosis, it is obvious that, if the survival period for carcinoma of the lung is only 10–14 months on the average as pointed out by Farber and Tobias,¹⁹ Tenzel⁹¹ and others, few patients at present have much hope for surgical cure by the time diagnosis is made.

There are many inherent difficulties in the diagnosis of bronchogenic carcinoma. Early in the course of the disease, the symptoms are minimal in severity and are very likely to be confused with "cigarette cough," bronchitis, etc. Overholt and Schmidt have reported that between 1942 and 1948 the average patient had symptoms for 3.8 months before seeing a physician and another 1.6 months passed before the first x-ray was taken. The usual diagnostic methods seemed to be applicable and effective only in the later stages of the disease for after the x-ray was taken, another 4.6 months passed before the diagnosis was finally made. There are a number of reasons for this last delay. Prominent among them is the fact that carcinoma of the lung simulates other diseases and accordingly, a positive diagnosis often cannot be made on the basis of clinical or x-ray evidence. A dependable working diag-

nosis based upon morphologic evidence is highly desirable, since most thoracic surgeons prefer some explicit knowledge of the lesion with which they must contend. Morphologic diagnosis in the past has required bronchoscopy, biopsy of lymph nodes, aspiration biopsies, or exploratory thoracotomy. These methods are not office procedures and from the standpoint of expense, comfort, and convenience, they are seldom undertaken on patients with minor symptoms. The doctor and the patient are rarely willing to subject themselves to these diagnostic measures until more severe cough, hemoptysis, pain, abscess, effusion, or other serious manifestations of disease have occurred. Moreover, these methods of obtaining tissue for diagnosis are not likely to be effective until the lesion is at an advanced stage. If extension to a lymph node which may be biopsied has occurred, there is no hope for the patient; aspiration biopsies are often dangerous, and not always successful according to many observers. Thoracotomy must be considered a major procedure.

The most important and effective means of securing tissue for morphologic diagnosis is by means of bronchoscopy, but

here again many bronchoscopists will assert that by the time the lesion is sufficiently large and prominent enough for bronchoscopic biopsy it has probably exceeded the bounds which limit resectability and cure. Clerf and Herbut¹² for instance reviewed 336 cases of cancer observed at the Bronchoscopic Clinic of the Jefferson Hospital from 1930 to 1940 and found that relatively few of the cases in which a positive bronchoscopic biopsy was secured were suitable for treatment. They stated that the percentage of positive bronchoscopic biopsies is in direct proportion to the number of inoperable cases.

It is evident that methods which have been available for morphologic diagnosis are such that they are seldom ordered before the disease has had a chance to advance beyond the stage of complete resectability. It follows then that some method which can be utilized in the presence of minimal thoracic signs almost as a routine procedure with relatively little expense and inconvenience to the patient and doctor is urgently needed to provide morphologic data. It is the purpose of this monograph to present such a method for discussion.

CHAPTER II

EVOLUTION OF CYTOLOGIC TECHNIQUE

MANY clinical manifestations of bronchogenic carcinoma are similar to those of tuberculosis, lung abscess, bronchiectasis, pneumonia, and other pulmonary diseases. Carcinoma of the lung often simulates these diseases, and they frequently take on the appearance of pulmonary carcinoma. Because of the difficulties in distinguishing cancer from benign conditions of the lung and bronchi, a certain diagnosis of bronchogenic carcinoma can hardly be made unless it is based upon morphologic evidence.

Beginning about one century ago there have been sporadic attempts to obtain morphologic evidence of lung cancer by demonstrating malignant cells in the sputum. In 1843 Walshe⁹³ found gross particles of cancer expectorated from the respiratory tract and in 1860 Beale⁵ found malignant cells in the sputum from a case of carcinoma of the pharynx. Subsequently until the beginning of this century, it was occasionally reported that cancer cells could be recognized in the sputum, but such findings were regarded as infrequent happenstance and were not thought to have much diagnostic significance. In recent years the increased awareness of bronchogenic carcinoma, and the hope for cure held forth by skillful thoracic surgery have greatly stimulated interest in all diagnostic measures, including examination of sputum for malignant cells, which might lead to the recognition of lung cancer.

Studies of cytologic techniques have fallen into 2 phases. The first involved investigations to discover how sputum and subsequently bronchial aspirations might best be prepared, fixed, and stained in order to demonstrate malignant cells in these materials. The second phase, based upon proof that malignant cells could be identified in the sputum, has been concerned with the evaluation of cytologic techniques for their applicability as practical diagnostic measures in the detection of pulmonary cancer. Throughout investigations in both phases, many criteria by which malignant cells might be identified have been established.

DEVELOPMENT OF THE CYTOLOGIC TECHNIQUE

Numerous investigations have been conducted to find practical methods for demonstrating malignant cells in the sputum and bronchial aspirations. Two different procedures have been utilized for basic preparation of the material: 1) study of sections made from "buttons" of sputum or bronchial aspirate by ordinary histologic techniques; 2) study of the smears of the sputum or bronchial secretions. The latter procedure has been preferred by most investigators.

SECTION TECHNIQUE.—Familiarity with routine histologic techniques has caused many pathologists to concentrate their efforts on fixing, blocking and sectioning

sputum or bronchial aspirations followed by staining with ordinary preparations in order to study malignant cells. Early workers with this technique included Sternberg,⁹⁰ Homan,⁴⁷ and Oberndorfer.⁷³ From South America, Palacio and Mazzei⁷⁷ as well as others have published enthusiastic reports on their work with sectioned material. Hunter and Richardson⁴⁸ in 1947 also reported excellent results from application of this technique. These investigators have claimed that sectioned material affords greater concentration of cells, finer cytologic detail, preservation of cell groups, and in addition that the method has the advantage of being a familiar one to technicians and pathologists. Other reports on the use of this procedure, however, have been less enthusiastic and disadvantages have been pointed out. The preparation of sections takes much time and care; cells may be distorted during sectioning; suspicious portions of the sputum cannot be chosen and thus serial sections must be made for adequate sampling. It is understandable, therefore, why this procedure has not been widely used.

SMEAR TECHNIQUE.—Hampeln in 1887,³⁸ using unstained smears of fresh sputum, was first to report finding malignant cells in the sputum from a proved case of lung carcinoma. He observed numerous large polymorphous cells in his smears and concluded that these could have arisen only from carcinoma. Other workers also studied unstained smears or smears stained according to simple methods. By use of these methods, grossly abnormal cells, particularly if present in clusters, could be recognized as malignant. In spite of the fact that it is necessary to study the details

of nucleus and cytoplasm to recognize less bizarre neoplastic cells, Hampeln³⁸ was able to report in 1918 on the detection of cancer cells in the sputum of 13 out of 25 cases of lung cancer, and in 1939 Althayzen² reported from Russia that he found 70 per cent of his cases of bronchogenic carcinoma by using this method.

Subsequent to the work of those mentioned above, much effort has been concentrated on the use of various fixatives and stains in order to bring out finer cytologic detail for study.

Bezancon and DeJong⁷ were the first to report on the application of rapid fixation to wet films of sputum. Their technique consisted of fixing wet films in a 1 per cent solution of chromic acid followed by staining with Unna's polychrome blue. They described the non-malignant cellular elements, discussed their origin, and compared their appearance with that of malignant cells. This technique was accepted and said to be useful by several European writers, including Dargallo¹³ of Spain and von Hooselin⁴⁶ in Germany. However, Ridge and Treadgold⁸⁵ of England (1913), studied the cytology of sputum smears made by the same technique and remarked that the stain was uncertain and that the films were impermanent.

Dudgeon, at the University of London, was impressed by the preservation of cytologic detail in parasites found in fresh smears of feces rapidly fixed in Schaudinn's solution and stained with standard preparations such as hematoxylin and eosin. He first utilized this method of fixation for cytologic studies of breast secretions in various pathologic conditions. In 1927, Dudgeon and Patrick¹⁷ applied this wet