Endoscopic Collection of Neoplastic Cells and Tubercle Bacilli from the Bronchi

(Description of a New Irrigation-suction Collector and the Anatomical, Physiological, and Pathological Factors Involved)

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The problem of early diagnosis in pulmonary disease constantly confronts the diagnostician and only too frequent are the pleas in the literature to send cases to the surgeon or the phthisiologist more promptly. Unfortunately the pathogenesis of both pulmonary neoplasms and pulmonary tuberculosis is of such a nature that, in spite of mass surveys, lesions are not always followed because of their apparent harmlessness or confusion with benign states. On the other hand they may appear after a negative report has reassured the patient that all is well. The flare for mimicry of both these entities as well as the speed or tardivity with which they progress is at times discouraging.

The far-advanced case is in reality no problem since the lesion is usually accessible to standard diagnostic procedures or the clinical picture is such that it is almost impossible to miss the diagnosis. On the other hand, the prognosis and the jeopardy of surgery are proportionately increased. Too often a lesion presents itself which may tax the ingenuity of all concerned by its inaccessibility to the usual methods of diagnosis and an operation may be recommended on a supposition with the subsequent gross loss of tissue and vital capacity. Only too often a lung removed for a neoplasm reveals silicotic nodules, tuberculomata, calcified foci or benign lesions of one type or another. Unless a proper diagnosis is made, in the face of present-day cancrophobia, pneumonectomy may easily become the vogue on a mere supposition.

In spite of the fact that we are all aware that lesions of the chest are too frequently found in far-advanced stages or definitely misdiagnosed,6 the fault does not always lie with the physician or the patient. The general practitioner, who in most cases is the first contact for the patient in the medical echelon is trained to diagnose and treat in the face of symptoms and to refer for specialized studies once again in the face of symptoms. The pa-

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tient on the other hand waits for symptoms before presenting himself for examination. In pulmonary disease, however, we must assume as a basic principle that in most cases the presence of symptoms connotes advanced disease states. The answer to early detection of such disease states is perforce more frequent roentgen inspections followed by more specific methods of diagnosis as well as their more universal application.

Present-day Methods of Diagnosis of Diseases of the Chest

Our mainstay to date in the diagnosis of diseases of the chest, exclusive of physical signs, which again presuppose advanced disease, has been the roentgen ray with its ingenious applications: fluoroscopy, fluorograms, flat films, laterals, obliques, apical lordotic views, progress films, stereoscopic films and finally the laminograph. These are all excellent methods of diagnosis in spite of the fact that they merely, with the exception of the laminograph, represent a composite of normal and abnormal shadows projected unto a flat surface. The laminograph is a compromise projection in that only a thin section is projected but its chief disadvantage is that, while in theory it provides for excellent definition, in practice it is reserved for comparatively coarse interpretation of hidden cavities and good-sized endobronchial masses. Fortunately we have built up in the science of radiology a table of experience which places many of these composite shadows into a definite diagnostic pattern. We must consider the fact, however, that similar patterns do not always mean the same disease and hence on occasions resolve into a radiological pitfall.

Bronchoscopy has now gained broad advantages in the differential diagnosis of diseases of the chest; but here again we are limited to lesions which are in the bronchoscopic axis and hence amenable to biopsy. The bronchogram with the exception of the diagnosis of bronchlectatic states has its limitations in that it merely shows a blocked bronchus which may be caused by any lesion or foreign body. Aspiration biopsy may be revealing if the lesion is accessible to the needle, but only too frequently results in a traumatic pneumothorax without a diagnosis being made. Diagnostic pneumothorax is mostly used to determine whether the lesion is attached to the chest wall, the diaphragm, or whether it is part and parcel of the pulmonary parenchyma. Thoracoscopy may be effective if the lesion is peripheral. Exploratory thora
cotomy is comparable to exploratory laparotomy and has its technical restrictions to accessible lesions; otherwise it is tantamount to the actual operation of lobectomy or pneumonectomy.

In the diagnosis of pulmonary tuberculosis most of the methods, with the exception of the x-ray, are aimed at the recovery and
Identification of the tubercle bacillus for a positive diagnosis. Examination of the sputa either directly or by concentration methods is the easiest and most popular. Cases of paucibacillary tuberculosis, however, are frequently not diagnostable by this method. Guinea pig inoculations and culture methods give an increase in the yield of positives. Examination of the gastric residue increases the number of positive yields in cases of paucibacillary tuberculosis and can be used in children and non-cooperative patients. It is interesting to note that studies of gastric sediments for tubercle bacilli give many false negatives because of the inhibitory action of the acid, as well as pepsin and gastric lipase, on the viability of the tubercle bacillus making their use for guinea pig inoculation and culture ineffective. This action is proportional to the time of contact between the bacillus and the gastric juices. More accurate results are obtained by immediate use of the juice or by its neutralization. Studies of the presence of non-pathogenic acid-fast rods in gastric juice would be an interesting development and would better evaluate this test clinically. Skin tests are merely a manifestation of sensitivity and, though of epidemiological value to determine inoculation phases, per se do not make or constitute a diagnosis of clinical tuberculosis. The sedimentation rate has the disadvantage of being non-specific and is used in tuberculosis as an auxiliary diagnostic measure or to check the progress of the disease. Finally, there are certain lesions suspicious of tuberculosis which are watched by temperature control and progress films which make this procedure also a method of diagnosis.

Much as in neoplasms the early diagnosis of pulmonary tuberculosis is too well inculcated in medical minds to re-emphasize. Early lesions can be treated by simple procedures and arrest obtained without the economic loss and the necessity for gross surgical procedures which become in reality salvage operations.

Endobronchial Methods of Diagnosis of Diseases of the Chest

Review of the literature in diagnostic procedures in the diagnosis of diseases of the chest reveals an increasing effort to get closer and closer to the pathological unit of disease by methods of direct approach to the bronchial tree and its subdivisions. The first organized impetus came after Papanicolaou discovered that neoplastic lesions in the uterus exfoliated characteristic cells which, under the microscope and with trained interpretation, gave a pathognomonic picture as revealing as the tissue section. It was not long thereafter that the method was applied to the pulmonary system for detection of pulmonary neoplasms. The incidental findings of tubercle bacilli in the bronchial secretions by
Clerf and Herbut\textsuperscript{12} have added a similar method of diagnosis of pulmonary tuberculosis. An interesting corollary to the studies of Clerf and Herbut is the popularization of the method of bronchial lavage for recovery of tubercle bacilli. This method is apparently widely in use in South America\textsuperscript{5,8} and sporadically in use in the United States.\textsuperscript{2} The technique is relatively simple and consists of either inhalation or instillation by catheter, under surface anesthesia, of sterile saline into the tracheo-bronchial tree and its recovery by cough. Sediments are studied for tubercle bacilli by the usual techniques. The percentage of positive yields by this method as compared with the gastric sediment or other standard methods is revealing. The dangers of droplet infection of attendant personnel using this method is, however, not discussed in any of the papers encountered.

It would seem that the examination of the endobronchially obtained secretions is the best answer to the earliest possible recognition of pulmonary neoplasms which are not approachable by conventional methods. When positive, the results are as revealing as a tissue section. The facility of this method, its lack of danger, and the high percentages of positive results in controlled cases (82.4 to 92.5 per cent)\textsuperscript{13} should make it the procedure of choice. The use of simple sputa is comparable to the method used in pulmonary tuberculosis. It is rather coarse and requires concentration methods as well as time-consuming search for characteristic cells. Much as in tuberculosis it presupposes a large yield and hence a far-advanced lesion. This method, however, is not to be condemned as it has good use in surveys and for mailing of specimens to distant laboratories.

What has been said about the examination of the endobronchial secretions for neoplasms applies aptly to their examination for tubercle bacilli. The method is not proposed to the exclusion of standard methods but merely to offer a method of collection in the finer bronchi for the earlier detection of the tubercle bacillus. Present experience is enough to warrant the statement that early lesions do discharge recoverable bacilli and these have been found in cases where no roentgen evidence of disease was present until six to eight weeks after pathogenic tubercle bacilli were isolated. The cases cited had pulmonary hemorrhage as the indication for the study.

**Effects of Bronchoscopy on Pulmonary Tuberculosis**

The most common deterrent to exploration of the tracheobronchial tree in pulmonary tuberculosis has been the fear that the procedure would activate the disease. Another is the danger inherent in the bronchoscopy per se or to the anesthetic. Unfort-
unately there is still a wide-spread belief that simple bronchoscopy is a rather formidable procedure. Coates and Osborne have reported a series of 473 bronchoscopies in 233 patients at Trudeau Sanatorium with no roentgen evidence of increase of the disease in a two-month period following bronchoscopy as compared with a similar period prior to bronchoscopy. A parallel observation was made by McInabe, et al, who bronchosco ped 272 patients with pulmonary tuberculosis with an increase of the tuberculosis in only four patients and this was not considered serious. The author has had the same experience with many bronchoscopies on tuberculous patients with no detectable spread of the lesions. In proper hands the procedure of bronchoscopy itself should be devoid of danger. The real danger lies in the use of excessive amounts of surface anesthesia. Review of the literature on such deaths would indicate that these were distinctly attributable to excessive amounts of the surface anesthetic. The author has developed a technique and innovations of instrumentarium which permit him to use subposological doses of the local anesthetic (Pontocaine) with no ill-effects attributable to the drug whatsoever; and this in spite of the fact that no barbiturates were used to counteract the effects of the Pontocaine. The series to date consists of 478 bronchoscopies without selection.

Pulmonary Anatomy, Physiology, and Pathology in Relationship to the Collection of Tumor Cells and Tubercle Bacilli

Fortunately for the clinician the structure of the lung is such that there is continuity of its smallest subdivisions to the larger, and fortunately again the pathogenetic development of both the lesions of neoplasms and tuberculosis is such that at some time or other there is discharge of the products of these lesions into the normal channels. This discharge may occur earlier than is usually realized because of the delicate normal structures involved. In the case of neoplasms their very nature of growth results in ischemia with subsequent necrosis as well as attrition of the surface of the parent mass by the constant respiratory movements of the lungs. Foci from inhalation tuberculosis are usually formed in the subpleural area and eccentric to or around the bronchioles and blood vessels. Because of the inherent property of tuberculous foci to cascade and ulcerate, the wall of a small bronchus may become eroded and the contents of the lesion discharged into an air passage.

The normal excretory forces constantly at work in the lungs are all integrated for the purpose of forwarding foreign bodies, secretions, or exudates from their source into the progressively larger normal channels until expulsion from the tracheo-bronchial
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tree is possible. These normal excretory forces may be overcome by the size of the unit mass (foreign body) or by the greatly increased numbers of unit masses (pulmonary edema, pneumoconiosis, lipoid pneumonia). A study of the distribution of the sensory endings in the bronchi9 shows that they become more and more infrequent as the lower levels are reached. Cough usually begins when the offending mass has moved up out of the respiratory part of the lobe to the point where the receptors are more numerous.9 Jackson9 has demonstrated the easy fatigability of these endings when he made the observation that an irritant applied to the bronchial mucosa resulted in cough but when the irritant was not removed nor its intensity increased, the cough gradually subsided.

Offending particulate matter at the lowest levels of the respiratory system (alveoli) is removed by three mechanisms: First, through the open alveolar duct into the bronchioles and progressively larger air passages; secondly, by way of the superficial lymphatics which follow the first radicle of the pulmonary vein from the center of the primary lobe to its periphery and then course outward to join the subpleural plexus of lymphatics which in turn unite forming lymph vessels that empty into the hilar lymph nodes; thirdly, by way of the deep lymphatics which follow the course of the bronchial and vascular trunks towards the hilar lymph nodes.15

The excretory mechanism which concerns us in the recovery of tumor cells and the tubercle bacillus is the first. Regardless of where the break-through occurs, we must assume, because of the intrinsic basic structure of the lung, that it will occur in one of the finer subdivisions. Once this occurs the microscopic defense mechanism against foreign matter begins its operation. In the case of the cancer cells probably no definite organized defense occurs and phagocytosis plays a minor part since the cells are usually larger than 10 microns which is the size limit that a phagocyte may ingest.15 The slough lies in the lumen and may behave as an inert foreign body calling forth some phagocytes and secretions to form an exudate about it. When large enough the exudate irritates the mucosa and is removed by the mechanisms to be described. In the case of the tubercle bacillus, however, a definite, well-studied response is had. Polymorphonuclear leukocytes are called forth and begin ingesting tubercle bacilli as well as the products of caseation. In this they are helped by the mononuclear phagocytes of the blood as well as the alveoli and ducts. The entire ensemble, in addition to fluid elements, again forms an exudate to be expelled.15

Motion of the exudate is towards the ductus alveolaris, if the
origin is in the alveolus; or towards the larger channels if it is formed elsewhere in the open airways. The pneumodynamic force of contracting air captured distally to the exudate acts as an air-hammer against the exudate and forces it along the path of least resistance, cephalad. The spiral musculature which reaches almost to the alveoli\(^\text{17}\) shapes and molds the exudate to facilitate removal. As the exudate rises to the ciliary level (0.3 to 0.4 mm. bronchioles)\(^\text{17}\) the new force of ciliary action which is always towards the larger air-ways begins to work. Rhythmic variations in the size of the bronchi as well as the lubricatory action of the products of the goblet cells, beginning at 0.4 mm. bronchioles,\(^\text{15,17}\) add another phase of the excretory mechanism. As the exudate is propelled cephalad to bronchi of a larger size and greater sensitivity,\(^\text{9}\) the powerful expiratory effort of cough with its great increase in intrabronchial pressure exerts a pneumatic force against the exudate. This results in actual projection of the exudate from the bronchi like a bullet from an air rifle.

For our purposes it suffices to say that the mechanism of excretion from the lungs is effective unless overwhelmed, but its study also provides us with the fact that early disease may be present without the accompaniment of cough.\(^\text{9}\) Also that the pathogenesis of tuberculosis and neoplasms is such that tubercle bacilli and exfoliated cells may be found far down in the bronchial tree and in many cases free in the lumens where they are recoverable by endobronchial aspiration.

**Method of Use of the Irrigation-suction Apparatus**

In studies of the normal bronchial tree the gentle scraping action of the spiral tip was sufficient on some occasions to make possible recovery of almost complete microscopic sections of the mucosa with all its histological characteristics: cilia, cuticular border, intact ciliated cell bodies, as well as the intermediate and basal cells. This gentle trauma did not result in bleeding, though occasionally blood-tinged yields are obtained. The presence of the formed elements of the blood can be used as a measuring stick when comparing cell sizes. The admixture of these elements did not cause confusion. It is felt that the use of gentle trauma has on some occasions resulted in the recovery of cells which were more characteristic of the parent mass than those that had been exfoliated.

In actual use the apparatus is introduced under direct vision into the bronchial branch orifice desired until it stops. Gentleness, of course, is essential as the spurs and the bronchial walls at lower levels are proportionately delicate and haste or pressure would result in a perforation. Three cubic centimeters of sterile saline
are then introduced; the petcock of the syringe closed; and the entire apparatus moved gently in and out for about one centimeter without disengaging the apparatus from the bronchus being studied. A short pause is then necessary to permit the solution to flow along the spiral tip by capillarity and by the negative suction action of inspiration. This pause is also necessary to give time for the cells or tubercle bacilli to be scraped off the mucosa and floated before they are aspirated. The process is repeated until at least 10 cubic centimeters of saline have washed the bronchus. Too long a period must not lapse after each instillation of the saline as too great an amount of the solution will be sucked into the alveoli and not recovered. The usual yield with this apparatus will average from five to eight cubic centimeters with a loss of two to five.

By this method it is possible to irrigate any lobar bronchus or any of the approachable segmental bronchi either selectively or seriatim. In the latter it is advisable to have entirely separate apparatus for each segmental or lobar bronchus to avoid errors through contamination. This change as well as the proper labeling of the reservoirs permits the operator to fairly well localize a lesion which will, in all probability, be confined to the bronchus with the greatest yield of tumor cells or tubercle bacilli. Due consideration must be given, however, to the possibility of contamination of non-lesion bearing bronchi by aspiration of cells or bacilli from the parent lesion.

**Processing the Washings**

It will be noted that the apparatus is equipped with a conical reservoir which is much similar to the 15 millimeter centrifuge tube. This permits direct centrifugalization of the yield in the standard centrifuge without the necessity of transfer. Centrifugalization is accomplished for 20 minutes at high speed and the fluid portion decanted by simple inversion. The packed residue is then aspirated in a long medicine dropper and smears made from the droplets on five cover slips—fewer may be used depending on the amount of yield. In cases where the secretions are abundant standard slide procedures may be used. For the study of tubercle bacilli the entire yield is spread on a slide, fixed, and stained by standard methods. For guinea pig inoculation and culture, the uncentrifuged specimen is transferred to a sterile test tube and submitted to the laboratory.

For the Papanicolaou stain the cover slips are placed in a small brass basket grooved to receive the slips and fitted with a handle. The entire basket is passed through the solutions seriatim. Solution containers consist of ordinary stock six ounce powder jars
Figure 1

(a) Straight, flexible tip collecting tube.
(b) Curved, flexible tip collecting tube.
(c) Collecting head.
(d) Reservoir.
(e) Cuff. Insufflation insert.
(f) Extra reservoir and closure.
with enameled tin screw covers. These are inexpensive and available at all drug stores. They are more effective in preventing evaporation between use. On completion of the stain, the cover slips are mounted on slides with Canada balsam. Usually a rather thin specimen is had by this method which makes possible good study without the cellular super-imposition seen on thick smears.

The Irrigation-suction Cell and Tubercle Bacilli Collector (Figure 1)

The principles followed in the design of this collector were those of complete visibility in the bronchoscopic axis and the possibility of almost simultaneous irrigation and suction through a tube with an external diameter of two millimeters which permits one to enter deeply into the tracheo-bronchial tree. In the development of this collector those designed by Clerf, Lukens and Tucker were thoroughly studied and one must admit that the basic principle and concept are not original. The improvements are. This collector will enter a 3 mm. x 45 cm. bronroscope though no visibility is had with the collector in situ. Vision is obtained through the bronchoscope without the collector; the bronchus to be studied engaged; and the apparatus introduced blindly. With the 5 mm. x 45 cm. bronroscope good vision is had with the apparatus within the bronchoscope and the bronchi entered with the usual techniques.

The Collecting Tubes

The collector is equipped with two tubes, one with a straight and the other a curved flexible tip. The actual length of these tubes is 53 cm. but the working length is 50 cm. This length permits working with 45 cm. bronchoscopes with still considerable lee-way in penetrating deeply into the lower respiratory passages. The external diameter of the tubes is 2 mm. which is a distinct advantage in probing bronchi of approximately this size. A lazy "S" design to the proximal end permits the tube to lie on the floor of the bronchoscope with no impediment to the line of vision. This end is also provided with a knurled taper swivel which permits rotation at any point of a 360 degree circle with positive locking and no compromise to the line of vision.

The Collecting Head and Reservoir

The collecting head has a "T" channel, the long arm of which communicates directly with the reservoir chamber. One portion of the "T" provides attachment by the taper swivel lock to the collecting tube and the other portion provides an air and watertight taper lock for the irrigating insert.
The Irrigating Insert

This consists of a stainless steel tube of small diameter with spring temper. Its length is such that when inserted into the collecting tube it comes to within 1 cm. from the proximal attachment of the spiral. It was found that any greater length would compromise the lumen of the spiral insertion as this is somewhat smaller than the diameter of the collecting tube proper. On the proximal end the irrigating channel is equipped with an air and water-tight taper lock for attachment to the head of the collector. A small stop-cock was added to close off the syringe from the system. Otherwise the syringe would be emptied when suction was applied. A female luer-lok attachment completed its assembly. This permits the use of standard Luer-Lok syringes. The author uses a 10 cc. syringe though a larger or smaller one may be used depending on what is desired.

For cases in which the secretions are abundant and the irrigation insert is not necessary, a closure for the irrigation channel port is provided with the apparatus. In this manner the apparatus may serve a dual purpose. With the use of the taper swivel locks the apparatus becomes a rigid and durable assembly, which can be manipulated with one hand. Wear on the tapers is immaterial inasmuch as any degree of tightness may be effected by tightening the knurled lock.

Other Uses for the Irrigation-suction Apparatus

In spite of the fact that this apparatus was primarily designed for the better collection of neoplastic cells other uses at once became obvious. The collection of tubercle bacilli from questionable lesions became so important that its use for this purpose was incorporated in this paper. The apparatus may be used for the detection of the etiological agents of mycotic diseases of the lung, clinical research in occupational dust inhalations, irrigation of both bronchiectatic areas or pulmonary abscess, as well as the instillation of antibiotics or other medications into the lower subdivisions of the bronchial tree.

The irrigation-suction collector for tubercle bacilli and neoplastic cells is being manufactured by the George P. Pilling & Sons Company, 3451 Walnut Street, Philadelphia, Pennsylvania, to whom the author has given the specifications for the manufacture of this instrument.

SUMMARY

1) The endoscopic approach to the positive diagnosis of pulmonary neoplasms and tuberculosis is discussed and compared to standard methods.
2) The anatomical, physiological, and pathological mechanisms
concerned in the recovery of neoplastic cells and tubercle bacilli are described.

3) A new irrigation-suction cell and tubercle bacillus collector which permits collection from lower subdivisions of the bronchi is described in detail.

4) The apparatus described permits either selective or seriatim irrigation and suction of the lobar bronchi or of the approachable segmental bronchi making possible diagnoses in a more limited anatomical area.

RESUMEN

1) Se discute y se compara con el método estandar la vía endoscópica para el diagnóstico de los neoplasmas y la tuberculosis.

2) Se describen los mecanismos que intervienen en la obtención de celdillas neoplásicas y bacilo de la tuberculosis.

3) Describese en detalle un nuevo aparato colector de bacilos y de celdillas de las subdivisiones más bajas de los bronquios.

4) El aparato descrito permite ya sea irrigación selectiva o seriada y succión de los bronquios lobares o del segmento investigado haciendo así posible el diagnóstico topográfico limitado de las lesiones.

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