DISEASES of the CHEST

VOLUME XXXIII  APRIL, 1958  NUMBER 4

An Aerosol Method of Producing Bronchial Secretions in Human Subjects: A Clinical Technic for the Detection of Lung Cancer

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The increasing importance of lung cancer as a major health problem has recently been emphasized by Gerhardt, Handy and Ferber who compared the age-standardized incidence rates for cancer for the years 1942 and 1955 in New York State. In man, the outstanding change in incidence rates over the 14 year period was the 202 per cent increase in carcinoma of the lung and bronchus. Even more striking is the increase of 520 per cent in the death rate due to lung cancer among the male population over the past 22 years. In women, this increase was 75 per cent. Similar trends have been reported for other states and for the United States as a whole.

The rising incidence of bronchogenic carcinoma makes early diagnosis a factor of vital importance. The number of patients diagnosed with lung cancer in an operable stage and with a reasonably good prognosis is still small. Lutwyche in a review of 116 cases of bronchial carcinoma noted a considerable delay in diagnosis in one third of his series. At present, there is no unanimity of opinion regarding the value of chest roentgenograms in screening for early lung cancer. Opinions vary from the belief that most cases have already metastasized by the time they are suspected radiologically, to the conviction that many are still localized and resectable.

Since the first report by Hampeln in 1918 on the presence of malignant cells in the sputum of patients with bronchogenic carcinoma, a number of investigators have confirmed the importance of cytologic examination in the diagnosis of lung cancer particularly in the early stages. Employing technics developed by Papanicolaou, the accuracy of diagnosis by sputum cytology has ranged from 84 to 93.5 per cent in the various series published. Farber and his associates reported a 90 per
cent accuracy in the positive diagnosis of lung cancer when five or more sputum specimens were examined. This degree of accuracy declined sharply when fewer specimens were studied.

In formulating a screening program for the early detection of lung cancer in apparently healthy individuals, cytologic methods have been considered impractical since the vast majority of these subjects are asymptomatic and raise no sputum. An approach to this problem has been made by utilizing the principles and technic of producing sputum in human subjects by the inhalation of hypertonic saline aerosols. The preliminary results of such cytologic screening are reported in this paper.

Theoretical Considerations

The problem of depositing an aerosol on the tracheo-bronchial mucosa which would induce bronchial secretions in apparently healthy individuals and yield sputum specimens suitable for cytologic study raised certain considerations. Among the factors which determine the degree of retention of an aerosol and the sites of deposition within the respiratory tract are: particle size and density, vapor pressure, temperature and hygroscopic properties of the aerosol, and the rate and depth of respiration. Findeln11 showed that 97 per cent of solid particles of 1 micron radius and above are deposited within the lung. Particles of 3 micra in radius are taken out completely by the trachea, bronchi and bronchioles. The retention of aerosol droplets in the human respiratory tract as a function of particle radius and respiratory rate was studied by Wilson and La Mer.12 A maximal retention of approximately 50 per cent occurred in the alveoli and bronchioles with particles of 1.0 micron in radius. The stability of a water droplet 0.5 micron in radius under ordinary conditions of humidity is only a fraction of a second due to its high evaporation rate. Furthermore, rapid absorption of isotonic aerosols took place within the tracheobronchial tree. Studies by Abramson13 indicate that droplet evaporation is decreased with aerosols in a glycerin solution because of the reduction in vapor pressure, serving to stabilize the aerosol mist.

| TABLE I |
| AGE AND SEX DISTRIBUTION OF 336 PATIENTS RECEIVING HYPERTONIC SALINE AEROSOLS |

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Sex</th>
<th>Number of Subjects</th>
<th>Age Mean (Years)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Male</td>
<td>125</td>
<td>55.3</td>
<td>21-90</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>78</td>
<td>53.1</td>
<td>18-86</td>
</tr>
<tr>
<td>B</td>
<td>Male</td>
<td>75</td>
<td>59.3</td>
<td>35-78</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>35</td>
<td>44.3</td>
<td>20-73</td>
</tr>
<tr>
<td>C</td>
<td>Male</td>
<td>20</td>
<td>58.9</td>
<td>30-75</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3</td>
<td>51.0</td>
<td>50-52</td>
</tr>
</tbody>
</table>

* A—No evidence of pulmonary pathology  
B—Chronic non-tuberculous pulmonary disease  
C—Known or suspected malignancy
Using aerosols containing radioactive solium, Talbot, Quimby and Barach\textsuperscript{14} found that the addition of 10 per cent propylene glycol to the aerosol solution results in a greater deposition within the lung than is produced by isotonic mists. Barach, Garthwaite and Bickerman\textsuperscript{15} noted that the comfort of inhaling penicillin aerosols is enhanced by providing a warm aerosol, and in addition, higher blood levels are obtained as compared with cold aerosols indicating an increased deposition of the penicillin mist.

The osmotic effect of hypertonic saline solutions for the purpose of this project was suggested by a study of Barach, Mason and Jones\textsuperscript{16} who noted a marked and instantaneous increase in plasma volume following the intravenous administration of 100 ml of 15 per cent saline solution. It seemed likely that the impingement of an adequate number of hypertonic saline droplets upon the bronchial mucosa would, osmotically, induce transudation. Such a bronchial transudate might then wash exfoliating cells from the regions of aerosol deposition into the material subsequently expectorated.

Methods

With the above considerations in mind, various concentrations of saline solution ranging from 3 to 15 per cent were employed to induce sputum production. A 10 per cent sodium chloride solution has been used in the majority of instances since the incidence of irritation appeared to be less than with the 15 per cent concentration. It was necessary to limit the saline aerosol to 3 per cent in some patients with bronchial asthma and pulmonary emphysema since the more hypertonic solutions appeared to accentuate obstructive dyspnea. Initially, 40 per cent propylene glycol

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.jpg}
\caption{Photograph illustrating the inhalation of warm hypertonic saline aerosol generated in a high flow nebulizer by means of an air pump.}
\end{figure}
was employed but this hygroscopic aerosol was too viscid and often occluded the nebulizer. The addition of 15 or 20 per cent propylene glycol to the saline solution provided a stable mist which did not usually interfere with continuous nebulization.

Since nebulization of a large quantity of aerosol solution within a limited period of 10 to 20 minutes appeared desirable, high-flow nebulizers which produced a relatively large particle size ranging between 1 to 10 micra in radius were employed. A small pump producing an air-flow of 5 to 7 liters per minute through the nebulizer generated the aerosol mist. Since initial studies had clearly shown that irritation was reduced and the comfort of inhalation enhanced by warming the aerosol, the solution within the nebulizer was heated by placing the reservoir of the nebulizer over a steam generator or electric coffee percolator.* The nebulizers originally employed included a large capacity baffle-less Vaponefrin nebulizer** and the Aero-Mist nebulizer.† More recently, a high-flow nebulizer‡ has been developed which nebulizes 1 ml. of hypertonic saline per minute, and incorporates a thermostatically controlled heating unit adjusted so as to provide an aerosol warmed to 105° to 130° C. A photograph and line drawing of this nebulizer are illustrated in Figures 1 and 2.

Procedure

A total of 336 patients from the wards of the Goldwater Memorial Hospital, Francis Delafield Hospital and the Asthma Clinic of the Co-

<table>
<thead>
<tr>
<th>Group*</th>
<th>Number of Subjects</th>
<th>Number Positive**</th>
<th>Positive Per Cent</th>
<th>Negative Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-I</td>
<td>180</td>
<td>155</td>
<td>86.1</td>
<td>25</td>
</tr>
<tr>
<td>II</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>B</td>
<td>110</td>
<td>99</td>
<td>90.0</td>
<td>11</td>
</tr>
<tr>
<td>C</td>
<td>23</td>
<td>23</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>336</td>
<td>277</td>
<td>82.5</td>
<td>59</td>
</tr>
<tr>
<td>Corr. Total (excl. A-II)</td>
<td>313</td>
<td>277</td>
<td>88.5</td>
<td>36</td>
</tr>
</tbody>
</table>

*—No pulmonary pathology (A-II pts. with impaired cough reflex)
B—Chronic non-tuberculous pulmonary disease
C—Suspected malignancy

**—mucoid sputum exceeding 1 ml.

*West Bend Aluminum Co., West Bend, Wisconsin.
**Manufactured by Vaponefrin Co., Upper Darby, Pennsylvania.
†Obtained from Medical Specialties, Inc., Boonton, New Jersey.
‡Manufactured by O. E. M. Corp., East Norwalk, Connecticut. Homemakers’ Products Corp. manufactures an inexpensive, effective apparatus providing the higher temperatures now generally employed.
Lumbia-Presbyterian Medical Center participated in the study. They were divided into three groups:

*Group A:* This group consisted of 203 patients with a wide variety of illnesses but with no evidence of pulmonary disease by clinical and roentgenologic study. These individuals were classified as apparently healthy with respect to the respiratory system and had no cough or spontaneous sputum.

*Group B:* This group consisted of 110 patients with chronic non-tuberculous pulmonary disease in whom the diagnosis of bronchial asthma, pulmonary emphysema and chronic bronchitis predominated.

*Group C:* This group consisted of 23 patients with known or suspected malignancy involving the lung or other organs. These patients were selected in such a manner that neither the technician who performed the test nor the pathologist who examined the sputum were aware of the diagnosis. Certain statistics for each of the three groups are shown in Table I.

Each subject inhaled the warmed aerosol produced by the continuous nebulization of a solution containing 10 or 15 per cent sodium chloride and 20 per cent propylene glycol for periods of 10 to 20 minutes. Because of an increased incidence in wheezing and coughing among the patients in Group B with bronchopulmonary pathology, the salt content of the aerosol was subsequently lowered to 3 per cent, as mentioned above, and was often preceded by an inhalation of 0.2 ml. of 2.25 per cent racemic

![Diagram of high-flow nebulizers with a thermostatically controlled heating element. Aerosol particle size ranges between 1 to 10μ in radius.](https://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21308/)
epinephrine (Vaponefrin)* or 0.4 per cent isuprel.** The latter procedure was followed in the last 20 patients of this group. All of the material expectorated during, and for 10 minutes following the aerosol inhalation was collected and measured. Gross appearance with respect to color, consistency and the presence of mucoid or muco-purulent shreds or plugs were noted. The recovery of mucoid material exceeding 1 ml. in amount indicated, in our opinion, that the specimen had been obtained from the tracheo-bronchial passageway since it could readily be distinguished from saliva, especially after the expectorated material was added to an equal volume of 70 per cent ethyl alcohol.

Spontaneous sputum collections were performed on 16 patients with chronic bronchitis prior to aerosol therapy. Each subject was then given an inhalation of 10 per cent sodium chloride and 20 per cent propylene glycol. Specimens obtained prior to the aerosol and after the inhalation of the hypertonic saline solution were analyzed quantitatively for total chloride content.

Sputum specimens following aerosol therapy were collected from 74 subjects including 19 patients with suspected malignancy, placed in an equal volume of 70 per cent ethyl alcohol and processed for cytologic study.

**Results**

Following the inhalation of hypertonic saline aerosols, specimens of spu	ta were obtained from 277 of the 336 patients treated. This is illustrated in Table II. Initially it was observed that 76.4 per cent of the patients in Group A yielded sputum specimens whereas 90 per cent of the patients with pulmonary disease and 100 per cent of the group with suspected malignancy raised adequate sputum samples. On further examination of the negative responders in Group A, it was found that 23 of these

<p>| TABLE III |
| DURATION OF INHALATION AND AMOUNT OF AEROSOL ADMINISTERED TO 277 SUBJECTS WITH RECOVERABLE SPUTUM |</p>
<table>
<thead>
<tr>
<th>Groups*</th>
<th>No. of Subjects</th>
<th>Per Cent Concentration</th>
<th>Aerosol Admin.</th>
<th>Sputum Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Saline Aerosol</td>
<td>Amount (Ml.)</td>
<td>Time (Ml.)</td>
</tr>
<tr>
<td>A</td>
<td>155</td>
<td>10 or 15</td>
<td>7.7</td>
<td>15.0</td>
</tr>
<tr>
<td>B-I</td>
<td>79</td>
<td>10</td>
<td>9.2</td>
<td>12.0</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>3</td>
<td>8.8</td>
<td>11.7</td>
</tr>
<tr>
<td>C</td>
<td>23</td>
<td>10</td>
<td>10.3</td>
<td>13.0</td>
</tr>
</tbody>
</table>

* A—No pulmonary pathology
B—Chronic non-tuberculous pulmonary disease
(B-II—asthmatics receiving 3 per cent saline)
C—Suspected malignancy

**Isoproterenol, 0.4 per cent, was mixed with phenylephrine 2.0 per cent, supplied as a Nebu-Isophrin by Homemakers' Products Corporation, Englewood Cliffs, New Jersey.
patients had neurologic disorders with marked impairment of the cough mechanism. These included 16 patients with hemiplegia, three with poliomyelitis, two with muscular dystrophy and two with meningovascular syphilis. Correcting the data by excluding these individuals, (Group A-II), resulted in an 86.1 per cent recovery of sputum from patients in Group A and an 88.5 per cent recovery for the combined series.

The amount of aerosol administered and the duration of each treatment were roughly comparable in all three groups. This is illustrated in Table III. Minor variations were related to the different nebulizers and aerosol pumps employed in the early phases of the study. Although the amount of sputum recovered varied widely from subject to subject, in general, those in Groups B and C gave higher yields than the subjects with no apparent pulmonary pathology. It must be recognized that the amount of sputum collected was at best a rough estimate since admixture with saliva and aerosol made quantitative measurement impossible. Nevertheless a sufficient amount of material was available in almost every instance for cytologic study.

Preliminary studies with cold hypertonic saline aerosols resulted in frequent complaints of a sense of irritation to the upper respiratory tract as manifested by scratchiness of the throat, hoarseness and cough. This was materially reduced when the aerosols were warmed to 40 to 44° C.

**THE INCIDENCE OF UPPER RESPIRATORY IRRITATION CAUSED BY INHALING HYPERTONIC SALINE AEROSOLS IN 336 SUBJECTS**

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21308/)

**FIGURE 3:** The incidence of upper respiratory irritation caused by inhaling hypertonic saline aerosols in 336 subjects, illustrating a positive correlation between the concentration of saline and per cent increase in irritation.
The incidence of upper respiratory irritation for the warmed aerosol is illustrated in Figure 3. Only 10 per cent saline solutions were employed in the patients with suspected malignancy and none of these reported any irritative symptoms. It is evident that a positive correlation exists between the incidence of irritation and the degree of hypertonicity of the saline aerosol. As might be expected, the per cent of subjects experiencing irritation was higher for those in Group B with asthma and pulmonary emphysema than for the subjects in Group A when either 10 or 15 per cent saline was inhaled. With a warmed 10 per cent saline aerosol, only three (4.3 per cent) in Group A, with no evidence of pulmonary pathology, complained of irritation.

A comparison of the total chloride content of the sputum collected on 16 subjects in Group B before and after administering an average of 8 ml. of 10 per cent saline aerosol showed a slight but insignificant increase in the chloride content of the sputum following the inhalation of the hypertonic saline solution. This is illustrated in Table IV. These findings would appear to indicate an osmotic effect resulting in the rapid dilution of the hypertonic aerosol droplets by bronchial transudate.

Cytologic Studies

Sputum specimens obtained from 74 subjects following inhalations of 10 per cent saline aerosol were subjected to cytologic study. Since material was recovered from patients with a variety of pulmonary disorders as well as from individuals free of lung disease, the character of the specimens varied greatly. In general, the induced sputum of "normal" individuals contained little gross particulate matter and appeared pale, whereas specimens from patients with pulmonary pathology appeared more mucoid and in some instances contained fresh muco-purulent plugs.

I. Method of Processing Specimen: The specimens arrived in the laboratory in glass bottles containing equal volumes of 70 per cent alcohol which had been added to the material expectorated. The entire contents were centrifuged for 15 minutes at 1500 rpm and the supernatant fluid decanted. The superficial part of the sediment was transferred to glass slides with a small spatula and two to four smears were prepared. Routine preparation included one hematoxylin-eosin and one mucicarmine

<p>| Table IV |
|-----------------|-----------------|-----------------|
| COMPARISON OF THE CHLORIDE CONTENT OF THE SPUTUM COLLECTED FROM 16 PATIENTS BEFORE AND AFTER INHALING 10 PER CENT SALINE AEROSOL |</p>
<table>
<thead>
<tr>
<th>Number of Subjects</th>
<th>Mean Amount of Sputum (ml.)</th>
<th>Mean Chloride Content Mgs. Per Cent</th>
<th>S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Aerosol</td>
<td>16</td>
<td>3.8</td>
<td>197.0</td>
</tr>
<tr>
<td>—mean of 8.3 ml. 10 per cent saline aerosol administered—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After Aerosol</td>
<td>16</td>
<td>6.4</td>
<td>244.7</td>
</tr>
</tbody>
</table>
stain. The remainder of the sediment was fixed in Bouin's solution, embedded in paraffin in the autotechnicon and sectioned. At least one hematoxylin-eosin and one mucicarmine stained slide were routinely prepared.

II. Cytologic Observations: a) Preservation of cells: No distortion of cells which might be attributed to hypertonic saline were observed. There was no shrinkage or crenation. The nucleus and cytoplasm seem to be preserved in relative proportion. Nuclear chromatin pattern was distinct and the granular or vacuolar character of the cytoplasm was maintained. The cells did not appear altered as compared with their spontaneously ejected counterparts. Variation in cell preservation and staining qualities could be attributed to the vicissitudes of the autotechnicon and technician.

b) Cell types: Sixteen of the 74 specimens contained only superficial squamous cells and leukocytes. These were regarded as unsatisfactory for study since there was no evidence that any of the material came from the lower respiratory tract. Most sediments contained alveolar phagocytes with anthracotic pigment and rarely some hemosiderin or fat vacuoles. The phagocytes varied in numbers about as one would expect from different individuals. Even healthy subjects yielded phagocytes (all were city dwellers or workers) and often in considerable quantities. The presence of these cells was regarded as an index of the depth from which the specimen was obtained since they occurred in clumps, which would be an unlikely finding if they were in the trachea or pharynx.

Bronchial epithelial cells were less numerous than alveolar phagocytes.

**FIGURE 4**
Photomicrograph of the sputum smear of a subject with hypertension (Group A) illustrating clusters of columnar, ciliated bronchial epithelial cells of normal character.—**FIGURE 5**: Smear of the sputum obtained from a patient with severe bronchial asthma (Group B) showing bronchial epithelium distended with mucus. One slender cell presents a ciliated border. This amount of mucus is abnormal.
but were very well preserved. In general these cells showed less alteration than that observed in spontaneous sputum. Cilia were easily seen. This is illustrated in Figure 4. One to three nuclei with fine chromatin appeared at the apposing end of the cell and the cytoplasm was finely granular or vacuolated. The vacuoles sometimes contained mucicarmineophilic material. In the material from several patients bronchial epithelium distended with mucus was abundant. The photomicrograph (Figure 5) of a smear from sputum obtained from a patient with severe bronchial asthma reveals bronchial epithelium distended with mucus. Polymorphonuclear neutrophils and lymphocytes were readily identified. Occasionally eosinophils were abundant.

The abnormal cells were not numerous, occurring in six of the specimens. None were found in material from healthy individuals. The benign abnormalities took the form of strips of cuboidal cells resembling swollen alveolar lining cells such as one sees about the air spaces in a lung with thickening of the alveolar walls. There were also cuboidal squamous cells with relatively large nuclei resembling parabasal cells which may have represented squamous metaplasia in the bronchial tree. In this series, cancer appeared only as squamous carcinoma. The malignant cells occurred individually and in clumps. One patient produced pieces of cohesive tumor cells. Figures 6 and 7 show squamous carcinoma cells from the cell block and smear respectively on sputum recovered from a patient with known squamous cell carcinoma of the lung. A cluster of malignant cells is illustrated in Figure 8 recovered in the sputum of a patient with epidermoid carcinoma of the lung.

**TABLE V**

**RESULTS OF CYTOLOGIC STUDIES PERFORMED ON SPUTUM RECOVERED FROM 74 SUBJECTS FOLLOWING INHALATION OF 10 PER CENT SALINE AEROSOL**

<table>
<thead>
<tr>
<th>Group*</th>
<th>Number of Subjects</th>
<th>Positive for Malign. Cells</th>
<th>Abnorm. Cells Suspicious Mal.</th>
<th>Negative for Malign. Cells**</th>
<th>Unsatisfact. Specimen***</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>27</td>
<td>23</td>
<td>4</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>28</td>
<td>21</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>19</td>
<td>4</td>
<td>2</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>74</td>
<td>4</td>
<td>2</td>
<td>52</td>
<td>16</td>
</tr>
</tbody>
</table>

* A—No pulm. path. B—Chronic pulm. disease. C—Suspected malignancy
**Specimen contained well preserved respiratory epithelium
***Insuff. quantity, no respir. epith., or purulent debris

*See Figures on Opposite Page*

**Figure 6:** A sheet of squamous carcinoma cells showing hyperchromatic nuclei, large nucleoli and mitotic figures recovered from the sputum of a patient with a known squamous cell carcinoma of the lung (Group C, cell block).—**Figure 7:** Individual malignant cells from the same patient with squamous cell carcinoma. The nuclei are relatively large, irregular and hyperchromatic with scanty cytoplasm (smear).—**Figure 8:** Cell block on the sputum recovered from a patient with epidermoid carcinoma of the left lung (Group C) showing a small cluster of malignant cells having relatively large, dark irregular nuclei and scanty cytoplasm. Several cells are degenerated.
A summary of the results of the cytologic study is shown in Table V. Fifty-eight (78.5 per cent) of the 74 specimens were judged to be representative samples of material from the lower respiratory tract satisfactory for diagnosis. Of these six from the group with suspected malignancy contained abnormal cells. Sixteen specimens were regarded as unsatisfactory for study since they contained only superficial squamous cells and leukocytes.

It was of interest to analyze the cytologic reports on the sputum obtained from the 19 patients in Group C which had been carried out in a "double-blind" fashion. This is illustrated in Table VI. Of the eight patients with documented primary or metastatic carcinoma of the lung, five of the sputum specimens induced by aerosol were positive for malignant cells. There were no false negatives in this group. Seven of the eight with malignancy in other organs not involving the lungs had no abnormal cells in their sputum, and one specimen was unsatisfactory for study. Of the sputum samples in the three patients with suspicious pulmonary infiltration on x-ray, one contained abnormal cells suggestive of squamous cell carcinoma, one was negative (subsequently diagnosed as tuberculosis) and one was unsatisfactory. Although conclusions cannot be drawn from this small series, the results of the cytologic study yielded no false positive or negative findings in those patients in whom a diagnosis had been established.

Discussion

Of the various factors which have been considered in an effective screening program for lung cancer, those of special importance include: 1) simplicity of technic, 2) non-injurious character of the procedure, 3) acceptance of the method by the subjects tested so that repeat studies are readily available, and 4) adequate criteria for establishing the diagnosis in order that the number of false positive and negative reports be minimal.

**TABLE VI**

ANALYSIS OF CYTOLOGIC STUDIES ON SPUTUM RECOVERED FROM 19 PATIENTS WITH KNOWN OR SUSPECTED MALIGNANCY (GROUP C) FOLLOWING INHALATION OF 10 PER CENT SALINE AEROSOL

<table>
<thead>
<tr>
<th>Group C</th>
<th>Number of Subjects</th>
<th>Positive or Suspicious Mal.</th>
<th>Negative for Malignant Cells</th>
<th>Unsatisfactory Specimen*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Known Bronchogenic Carcinoma</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>2. Known Metast. Ca. to lung</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3. Suspected lung cancer</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4. Known or suspect. Ca. elsewhere</td>
<td>8</td>
<td>0</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>19</strong></td>
<td><strong>6</strong></td>
<td><strong>8</strong></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>

*purulent exudate predomin., no respiratory epithelium seen.
The recovery of sputum adequate for cytologic study in 88.5 per cent of the combined series of 336 subjects following a single treatment with hypertonic aerosols justifies the extension of this program to a larger series. It is quite possible that negative responders might be induced to raise sputum following a second or third course of treatment with saline aerosol. Further study is required to ascertain whether conversion of these negative responders would occur with higher concentrations of saline aerosol. In addition, the effect of mechanical coughing by exsufflation with negative pressure following the inhalation of the saline aerosol may assist in raising bronchial secretions from the smaller bronchi and bronchioles particularly in those individuals with a weak or ineffective spontaneous cough.

The various alterations in cell morphology and the many bizarre forms of respiratory epithelium observed on cytologic study of sputum from patients with chronic pulmonary disease have recently been reported by Papanicolaou,16 and Farber and his associates17 who emphasized the need for rigid criteria in differentiating apparently benign metaplastic changes from carcinoma. From these reports and others, there is some evidence that the bronchial epithelium reaction leading to malignancy may be manifest for some time before the clinical diagnosis of lung cancer is possible.

Cytologic examination of the 74 sputum specimens recovered following the inhalation of 10 per cent saline aerosol indicated that the majority of the specimens expectorated contained respiratory epithelium and alveolar phagocytes. Cellular morphology was well preserved so that the clear cut differentiation between abnormal and normal cells was readily apparent to the experienced pathologist. In addition to the value of this method of obtaining sputum for the cytologic diagnosis of lung malignancy, the importance of bacteriologic as well as cytologic examination of induced sputum deserves further emphasis, especially in those patients with undiagnosed respiratory disease from whom no spontaneous sputum is available for study.*

**SUMMARY**

A method of obtaining sputum from the respiratory tract of "normal" individuals, patients with chronic non-tuberculous pulmonary disease, and patients with known or suspected malignancy is reported. The basis of this procedure involves the inhalation of warm, hygroscopic saline aerosols.

*Since this paper was written significant developments in the principles and technics of aerosol deposition have been made. At temperatures of 120° to 130° F., 10 liters of air contain 0.185 gm H₂O, at 120° F., 0.789, at 140° F. 1.302 gm H₂O. Over a period of 30 minutes inhalation approximately 11 cc. of water would theoretically be deposited on the laryngo-tracheo-bronchial mucous membrane at 120° F., and 39 cc. at 140° F. In an aerosol administered at 98° F. the particles are preserved by the humidified atmosphere but significant enhanced "rain-out" effect takes place above body temperature. The clinical advantages of inhaling aerosols with controlled temperatures of 120° F. and 130° F. in patients with bronchial asthma, pulmonary emphysema, and a variety of respiratory disorders characterized by difficulty in eliminating retained bronchial secretions will be reported later. (Barach, Alvan L., Beck, G. J., and Bickerman, H. A.).
Approximately 10 ml. of a 10 or 15 per cent saline aerosol containing 20 per cent propylene glycol was administered to a total of 336 subjects. Mucoid sputum specimens suitable for cytologic examination were recovered in 88.5 per cent of the entire series. Of special interest as a possible screening technic for the cytologic diagnosis of lung cancer was the production of sputum in 86 per cent of 180 subjects who had no evidence of pulmonary disease, and no cough or spontaneous sputum. Only three subjects in this group noted a slight irritation on inhaling the warm 10 per cent saline aerosol.

Cytologic studies of the sputum recovered from 74 subjects following the inhalation of 10 per cent saline showed that 79 per cent of the specimens were satisfactory for diagnosis. In five of the eight patients with documented primary or metastatic carcinoma in the lungs, sputum preparations were positive for malignant cells. There were no false negative reports. Since all of the examinations were performed on single specimens, it is evident that cytologic study of repeated specimens obtained in a similar manner might increase the possibilities of accurate diagnosis. It is the opinion of our cytologist (E. E. S.) that this method of inducing sputum may be expected to demonstrate tumor cells from symptomless individuals before spontaneous sputum arises since the specimens obtained by hypertonic saline aerosol are entirely adequate for study.

Inhalation of nebulized 20 per cent propylene glycol at a temperature of 120° F. is employed to facilitate expectoration of retained secretions in patients with bronchopulmonary disease.

RESUMEN

Se describe un método para obtener esputo de las vías respiratorias de los individuos "normales" con enfermedad crónica no tuberculosa y enfermos sospechosos o averiguados de padecimientos malignos.

La base de este procedimiento radica en la inhalación de aerosoles calientes, de soluciones salinas higroscópicas.

Aproximadamente se usaron 10 ml. al 10 o 15 por ciento de un aerosol conteniendo 20 por ciento de propilen glicol en un total de 336 sujetos. Se obtuvo un especímen mucoide adecuado para estudio citológico en 88.5 por ciento de los casos.

Es de interés especial este método como uno para la detección del cáncer por la citología ya que se obtuvo esputo en 86 por ciento de personas entre 180 sujetos que no tenían evidencia de enfermedad ni tos ni expectoración. Sólo tres personas de este grupo notaron ligera irritación al inhalar la solución caliente al diez por ciento en aerosol.

Los estudios citológicos en el esputo de 74 personas después de la inhalación de la solución salina al 10 por ciento, mostró que 79 por ciento de los especímenes fueron satisfactorios para el diagnóstico. En cinco de ocho enfermos comprobados de carcinoma primario o metastásico las preparaciones de esputo fueron positivas para células malignas. No hubo informes falsos negativos. Puesto que todos los exámenes se hicieron en sólo una muestra, es evidente los estudios en muestras repetidas hechas.
en forma similar han de dar mayor proporción de diagnósticos exactos. El citologista opina (E.E.S.) que este método de provocar el esputo puede esperarse que muestre células tumorales de individuos asintomáticos antes de que obtenga esputo espontáneo ya que los especímenes fueron completamente adecuados para el estudio.

RESUME

L'auteur présente une méthode qui permet d'obtenir des crachats provenant de l'arbre respiratoire chez des individus "normaux," chez des malades atteints d'affections pulmonaires chroniques non tuberculeuses, et chez des malades atteints d'affections malignes, connues ou soupçonnées. La base de ce procédé comprend l'inhalation d'aérosols salins, chauds et hygroscopiques.

Environ 10 cc. d'un aérosol à 10 ou 15% de sel, contenant 20% de glycol propylen, a été administré à un nombre total de 336 malades. Des types d'expectorations mucoïdes propres à l'examen cytologique furent recueillis dans 88,5% de la totalité du groupe. La production d'expectoration dans 86% des cas, sur 180 malades chez lesquels on n'avait pu mettre en évidence une affection pulmonaire, et qui n'avait ni toux ni expectoration spontanée, fut d'un intérêt particulier, pour permettre le diagnostic cytologique de cancer pulmonaire. On observa chez trois sujets seulement de ce groupe une irritation légère lors de l'inhalation de l'aérosol chaud contenant 10% de sel.

Les études cytologiques sur l'expectoration recueillie chez 74 malades après inhalation de solution à 10% de sel, montrèrent que 79% des spécimens d'expectoration furent satisfaisants pour le diagnostic. Chez cinq malades sur 8 qui étaient atteints de cancer pulmonaire métastatique ou primitif prouvé, les expectorations montrèrent l'existence de cellules malignes. Il n'y eut aucun cas d'erreur. Comme tous les examens furent pratiqués sur un simple échantillon, il est évident que l'étude cytologique d'échantillons multiples obtenus dans des conditions semblables pourrait augmenter les possibilités d'un diagnostic précis. Notre cytologiste exprime l'opinion que cette méthode d'obtenir une expectoration pourrait amener à mettre en évidence des cellules tumorales chez des individus sans symptômes, avant qu'une expectoration spontanée ne survienne, étant donné que les échantillons obtenus par aérosol salin hypertonique se prêtent parfaitement à l'étude.

ZUSAMMENFASSUNG


Ungefähr 10 ccm einer 10-oder 15% Salz-Aerosol-Lösung, die 20% Propylen-Glycol enthielt, wurde insgesamt bei 336 Personen angewandt. Für die cytologische Untersuchung geeignete Schleimhaltige Sputumpräparate wurden in 88,5% der ganzen Serie gewonnen. Von speziellen Inter-
esse als eine mögliche Suchtechnik zur cytologischen Diagnose des Lungenkrabbes war das Zustandekommen von Auswurf bei 86% von 180 Personen, bei denen kein Anhalt für eine Lungenkrankheit bestand, also auch kein Husten und auch kein spontaner Auswurf. Nur 3 Personen aus dieser Gruppe bemerkten eine leichte Reizung bei der Inhalation der warmen 10%-igen Salz-Aerosol-Lösung.


REFERENCES
4 Davies, D. F.: Personal communication.