between viscosity and dry weight and neuraminic acid content and an inverse relationship to the degree of airways obstruction (Fig 1).

Analysis of bronchial casts in patients with status asthmaticus suggest two mechanisms of cast formation. In one there is gross dehydration of the mucus with dry weights over 250 mg per ml. In the other, the dry weight and neuraminic acid content is similar to that of the patient's mucoid sputum and cast formation may be due to alteration in electrolyte content.

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Discussion

Dr. Lyons: While the correlation in Dr. Chodosh's presentation between the ventilatory and the physical properties of the sputum was considered highly significant, it seemed to me low. Perhaps drugs were altering the properties of the sputum, while if not, perhaps altering the patient's symptoms and his ventilatory properties. The patients chosen for this study were in a double-blind drug study. I therefore have some doubts whether Dr. Chodosh's results had the consistency that is contended.

Dr. Chodosh: The correlation between the sputum and ventilatory capacity did exist independently of the drugs used.

Dr. Keal: I agree that the changes in the property of the sputum were not secondary to drugs, but were, in fact, spontaneous changes.

Dr. Middleton: Dr. Keal, is there a correlation between the neuraminic acid content and the number of cells present in mucoid sputum and have you looked for neuraminidase in the sputum?

Dr. Keal: We have not made any attempt to quantify the cell content of sputum and, therefore, I cannot answer the first part of your question. We have studied the neuraminidase content of the sputum because many organisms that attack mucusal surfaces produce neuraminidase. There may be an association between bacterial neuraminidase and the cellular neuraminic acid within the sputum.

Dr. Townley: In some of our in vitro studies, neuraminidase does not seem to affect the viscosity of sputum directly, but if sputum is pretreated with neuraminidase and then treated with a proteolytic enzyme such as trypsin, the viscosity would decrease markedly. Trypsin or chymotrypsin alone would not do this. This treatment seems to work on all asthmatic sputum.

Dr. Reed: Dr. Keal, does there seem to be any effect of Isuprel upon the viscosity of sputum?

Dr. Keal: I have not done this study, but others have found that isoproterenol increases the size of goblet cells.

Dr. Chodosh: Isoproterenol also seems to act as an expectorant and therefore actually aids in sputum removal.

Dr. Pepys: Is it possible to rehydrate dehydrated sputum in vitro?

Dr. Keal: It is, in fact, possible to rehydrate sputum in vivo by rehydrating the patient. Earlier workers have shown that a doubling of the fluid intake in such a patient could lead to a threefold decrease in sputum viscosity.

Biochemical Characteristics Affecting the Consistency of Bronchial Secretions

A. D. Barton, Ph.D.; J. L. Powers, B.S.; Melvin Lopata, M.D.; and Ruy V. Lourenco, M.D.

In patients with chronic obstructive pulmonary disease, the bronchial secretions are frequently abnormal in quantity, composition and consistency. The resulting sputum, with its altered viscoelastic properties, may be responsible in part for the impaired mucociliary clearance demonstrated in our
earlier experiments with inhaled aerosols. Furthermore, alterations in the secretions may interfere with the distribution of ventilation in the lungs. Recognition of early abnormalities in the bronchial secretions might aid in detecting airway disease while it is still reversible. Also, definition of abnormalities in the bronchial secretions may help in understanding the pathogenesis of chronic pulmonary diseases.

We have investigated various factors contributing to sputum consistency, including the organization of water in the sputum gel, the types of chemical bonding responsible for the gel structure of the bronchial secretions, and their alteration in purulent sputum.

Sputum often behaves like a semisolid: when placed in a tube with a constricted opening at the bottom, it will not flow through. Also, water added on top will not flow through the gel. The state of water in the gel is not entirely clear. Evidently enough of the water is organized around the glycoprotein structural elements to prevent flow through the gel. On the other hand, we have found that sucrose diffuses freely into at least 90 percent of the water in the gel without obvious alteration of the gel structure. The bronchial secretion consists almost entirely of water, yet it behaves like an elastic semisolid. It is probably no exaggeration to suggest that the purpose of the long glycoprotein molecules of the bronchial mucin is to organize water so it has viscoelastic properties appropriate for coating the airways and participating in mucociliary clearance.

The elastic semisolid consistency of the bronchial secretions implies cross-linking between the structural elements and we have studied the dispersal of sputum samples by agents that disrupt secondary bonds, in order to assess the contribution of these bonds to the sputum gel structure. Treatment with propylene glycol (final concentration 30 percent) dispersed mucoid sputum, eliminating its semisolid character. Evidently hydrogen bonding is one important factor in the sputum gel structure. However, the suspension still showed elastic recoil and spinnbarkeit (the ability to be drawn out in a liquid thread), and the individual components of the gel still were not free to migrate independently in cellulose acetate electrophoresis. Apparently the structural elements still were cross-linked by disulfide bonds because subsequent reduction with N-acetylcysteine eliminated the elastic recoil and spinnbarkeit and released the gel components. On the basis of these experiments it appears that the basic gel structure of the bronchial secretions consists of long glycoprotein molecules\(^2\)\(^3\) that are cross-linked by disulfide bonds to form a loose network. The elements of this network are additionally cross-linked to one another and to water molecules by hydrogen bonding to form a semisolid gel that presents elastic resistance to deformation.\(^4\) Whether the glycoproteins are disulfide bonded to one another directly, or through other protein components remains to be determined.

With increased purulence, the bronchial secretions often become dense and tenacious, and increasingly resistant to dispersal. In experiments studying the effect of purulence on the biochemical characteristics of sputum each specimen was first extracted with propylene glycol (final concentration 30 percent), centrifuged at 1,000 x g for one hour, and the supernatant containing the propylene glycol fraction was removed. The sediment was then extracted with N-acetylcysteine (final concentration 4 percent), centrifuged at 1,000 x g for one
Figure 2. Distribution of DNA among the three sputum fractions in relation to the total DNA content of the sputum. In mucopurulent sputum (right side) the deoxyribose nucleoprotein fibril material from disintegrated leukocytes appears to be cross-linked by disulfide bonds, since it resists dispersal by propylene glycol, but is dispersed by N-acetylcysteine.

Discussion

Physician: Have you looked for alveolar macrophages in the sputum?

Dr. Barton: We have been trying to localize the enzyme in the sputum by means of histochemical procedures, but our results so far are not conclusive.

Dr. Farr: Does all the DNA in the sputum come from cells within the sputum?

Dr. Barton: According to Potter, the DNA in the sputum has the composition of human DNA rather than bacterial DNA, and therefore comes from breakdown of host cells.

Dr. Lyons: Do most of the enzymes come from alveolar macrophages?

Dr. Barton: We have found the gamma-glutamyl transpeptidase in alveolar macrophages, but it may have been picked up by phagocytosis.

Dr. Chodosh: It is interesting that few alveolar macrophages are found in the sputum of bronchitics and increased number of macrophages within the sputum of patients is usually associated with a decreased viscosity of that sputum.

Dr. Macklem: What do these enzymes do to collagen?

Dr. Barton: This is unknown.

Dr. Hirsch: Sputum stains done with Bismarck brown for protein, toluidine blue for acid mucopolysaccharide, PAS for DNA, and fibrin stain for fibrin, all show high concentrations of each of these within the sputum. Emersion of Bismarck brown and toluidine blue stained sputum slides in acetyl cysteine illustrates that it dissolves the protein and acid mucopolysaccharide.

The three major constituents of sputum, aside from the actual sputum, appear to be mucopurulent material and nucleoprotein fibril material. Much of the material may resist the two extractions and remain in the final residue fraction, as shown on the right in Figure 1. The relationship between the total DNA content of the sputum and the distribution of the DNA among the sputum fractions is shown in Figure 2. Toward the right side of this figure we see that in mucopurulent sputum with high DNA content the deoxyribose nucleoprotein fibril material resists dispersal by propylene glycol and is dispersed by N-acetylcysteine, suggesting that it too is cross-linked by disulfide bonds. Thus, three factors seem to contribute to the abnormal coherence of mucopurulent bronchial secretions: increased disulfide bonding between the structural elements of the bronchial gel (more of the material resists dispersal by propylene glycol, but is dispersed by N-acetylcysteine); additional more stable bonding, possibly by covalent cross-linking (more of the material resists dispersal by propylene glycol, N-acetylcysteine and other secondary bond agents); and addition of doxyribose nucleoprotein fibril material from disintegrated leukocytes.

References


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from water, are DNA, acid mucoprotein and fibrin. Sputum mucoprotein structure can crudely be shown as disulfide and hydrogen bond linked proteins joined by calcium sulfate and ammonium bonds to a column of saccharides with a molecule of fucose on one end and sialic acid molecule on the other end. By far, the largest part of this molecule is its protein moiety which also is the most treatable part in that its disulfide bonds are ruptured by acetylcysteine. Further illustration of the usefulness of attacking the protein moiety of the mucopolysaccharide is the rapid lysis and decrease of viscosity of viscous sputum brought about by trypsin digestion.

Because of the relative unsuitability of previous methods in the clinical measurement of sputum viscosity, we have developed the following method which has been highly satisfactory for us. The fluid consisto-viscosimeter was designed in our laboratory to measure the consistency of heterogenous semi-plastic materials such as sputum. The device consists of hollow stainless steel plunger with a perforated disc at its lower end which is driven by a constant infusion pump through a close fitting barrel filled with sputum. The bottom of the cylinder is fitted with a transducer which reflects the pressure generated as the sputum is forced through the perforated disc. This pressure is directly proportional to the sputum consistency, which is expressed in "consistency units." The thicker the sputum, the greater the number of consistency units. One consistency unit is equivalent to 1,500 centistokes (the standard units of kinematic viscosity).

We have recently completed a double-blind crossover study of the effect of glyceryl guaiacolate on chronic bronchitis and have shown that it does not affect ventilatory capacity or the clinical course of the disease. Furthermore, glyceryl guaiacolate does not increase the ease of expectoration or alter the consistency or volume of the sputum. A similar double-blind crossover study, using potassium iodide, likewise showed no change in ventilatory capacity, nitrogen washout, or clinical course in patients with chronic bronchitis. Sputum consistency, sputum volume and ease of expectoration showed no consistent differences in patients given iodide or placebo. In summary, the most effective agents we have at this time for reducing sputum viscosity are N-acetylcysteine and water.

**Dr. Salvaggio:** Does N-acetylcysteine perhaps alter or destroy immunoglobulins in view of the fact that it ruptures disulfide bonds? Could it increase the chance for infection by altering "protective" secretary IgA for example?

**Dr. Hirsch:** I don’t know.

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**Bronchopulmonary Lavage in Bronchial Asthma**

*Robert M. Rogers, M.D., John F. Shuman, M.D., and Alan B. Zubrow, B.A.*

Airway obstruction in bronchial asthma during an acute attack is predominantly due to a bronchospasm. However, as the attack persists, edema of the bronchial mucosa and thick tenacious mucus in the airway lumen contribute to airway obstruction. The thick mucus may contribute to the morbidity and mortality of status asthmaticus by causing atelectasis which leads to severe hypoxemia. In addition, the mucus may serve as a depository of an antigen which continues to stimulate airways obstruction when it cannot be removed by cough or other means—ie, aspergillosis. The purpose of this report is to review our experience with bronchopulmonary lavage in seven asthmatic patients.

The technique of lavage has been described in detail elsewhere. Simply, it is accomplished by alternately filling and emptying one lung while the nonlavaged lung is used to maintain gas exchange.

Bronchopulmonary lavage in asthmatic patients offers several unique technical problems: 1) high airway resistance decreases flow of fluid so that the procedure takes considerably longer; 2) inhalation anesthesia induction is prolonged because of decreased gas exchange; 3) manipulation of the airway with a Carlen tube may induce further bronchospasm which must be treated in the context of lavage; 4) ventilation of the nonlavaged lung may require high pressure making adequate alveolar ventilation difficult to achieve, so that CO2 retention during the procedure is common; 5) more fluid remains in the lung at the end of lavage in asthmatics (mean = 1340 ml) than in patients with alveolar proteinosis (mean = 740 ml).

To obviate some of these problems, we usually give IV aminophylline three hours prior to lavage, give IPPB with a bronchodilator immediately before induction of anesthesia, apply local anesthesia to vocal cords and trachea, give IV aminophylline