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The Pneumococcus and Some Men Who Came to Yale: The Dorothy M. Horstmann Lecture[a]

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Yale has been fortunate indeed to have had Dorothy Horstmann as a member of its faculty for all but one of the last 50 years. It has had also the wisdom to take cognizance of her value as an individual and of her contributions to biomedical science and human welfare on two occasions in recent years. Her studies of poliomyelitis, hepatitis, and rubella, executed with perceptiveness, rigor and modesty, have benefited countless numbers; and for her many achievements all are in her debt. I am beholden to her colleagues for this opportunity to pay tribute to a wise and gracious friend.

In casting about for a subject befitting this occasion, the thought occurred that it might be of interest to examine the contributions of some former and present members of Yale's faculty to the subject of a group of infections still endemic in all human societies, namely those caused by *Streptococcus pneumoniae* or the pneumococcus. The list is doubtless not exhaustive but includes such notables as Winternitz, Blake, Paul, Trask, Eaton, and Beeson of former days, as well as reflecting ongoing investigations today by Eugene Shapiro and his colleagues. In reviewing some of this earlier work, it will be my endeavor to place it in the context of contemporary understanding. In the interest of some semblance of order, the material will be examined in topical rather than in chronological order, dealing with bacteriologic and immunologic, pathogenetic, therapeutic, and prophylactic considerations in that sequence.

Pneumococci were first isolated independently from human carriers of the organism in 1880 by Sternberg and by Pasteur [1]. Their role as a major incitant of bacterial pneumonia in man was demonstrated by Weichselbaum in 1886 [2]. Evidence of the heterogeneity of pneumococcal strains began to appear at the end of the 19th century and was established clearly by mouse protection tests reported by Neufeld and Händel in 1910. One of the curious paradoxes of bacteriologic history is that the Quellung reaction, discovered eight years earlier by Neufeld, was used neither by him nor by others in the next two decades [3]. All the work until the 1930's done to delineate the first 29 pneumococcal types was carried out with other immunologic techniques, such as mouse protection, agglutination, and precipitin tests.

One of the most memorable members of Yale's medical faculty who had a life-long interest in the pneumococcus and the infections it causes was Francis Gilman Blake [4]. Born in Pennsylvania in 1887, he grew up in New England, attended Dartmouth College, and received his M.D. degree from Harvard in 1913. After house officer training at the Peter Bent Brigham Hospital, Dr. Blake began his academic career as an assistant professor of medicine at the University of Minnesota in 1917, and that same year published the first of many papers dealing with the pneumococcus, one entitled "Methods for the Determination of Pneumococcus Types" [5]. At the time only three types had been delin-

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eated clearly, types 1, 2, and 3, but differences in prognosis among infections caused by the three types had been recognized, and their identification was essential if serum for therapy was to be employed. Blake discusses the importance of the proper collection of sputum and its microscopic examination, both still relevant today, the intraperitoneal inoculation of sputum into the mouse, and the employment of peritoneal washings from the mouse in agglutination and precipitin tests with type specific antisera, prepared apparently at the Hospital of the Rockefeller Institute. Use of the latter test took cognizance of the specific soluble substance of pneumococcus described by Dochez and Avery in March, 1917. The precipitin reaction had the advantage of providing a specific result when the presence of mixed bacterial flora in mouse peritoneal washings rendered agglutination unfeasible. It was not until 1933 that Neufeld and his collaborator, Etinger-Tulczynska, reported using the Quellung reaction to type pneumococci. Its use in this country followed publication in 1933 of a paper by Albert Sabin, who had learned the technique from Kenneth Goodner following the latter's visit to Neufeld's Laboratory [3]. The Quellung test became the standard method for typing pneumococci in diagnostic laboratories during the brief remaining period of serum therapy but is one employed rarely in hospitals today. It has been resurrected of late in sentinel laboratories in several parts of the world to ensure the appropriateness of the formulation of contemporary pneumococcal vaccines.

Blake published a second report on immunologic aspects of pneumococcal infection the following year entitled "Antigen-Antibody Balance in Lobar Pneumonia" [6].

Aware of the studies of Dochez and Avery that a "soluble pneumococcus substance" is present in the blood of some patients with severe lobar pneumonia and is excreted in the urine of a considerable number of cases, Blake set out to determine the relationships of this antigen to the earlier described antibody and whether or not these relationships might be of prognostic value. He was able to categorize three groups of cases: (1) mild infections with recovery and soluble pneumococcus substance undetectable in blood or urine, (2) severe cases with bacteremia and soluble substance in urine but not blood which recovered, and (3) fatal cases with bacteremia and increasing amounts of soluble substance in both blood and urine. Agglutinins appeared in the blood of patients in Group 1 usually about the time of crisis but were absent from the blood of patients in Group 3.

In the course of redeveloping pneumococcal vaccine, the immunologic phenomena occurring in the course of bacteremic infection were reexamined quantitatively in the 1970s by means of a radioimmunoassay [7]. The levels of antibodies in a patient recovering from bacteremic type 4 pneumococcal pneumonia rose sharply in the month following onset of infection, then fell to approximately half their peak value over the next six months where they remained over the course of the next three years. Pneumococcal capsular polysaccharides appear not to be degradable by mammalian enzymes and may be detected by the use of immunofluorescent antibodies in the lymph nodes months after their injection into experimental animals [8]. Both findings are consistent with the observation that recurrent infection of man with the same pneumococcal capsular type occurs rarely, if ever, save in the agammaglobulinemic or dysgammaglobulinemic patient. The radioimmunoassay can be used also to demonstrate the presence of circulating capsular polysaccharide in patients with bacteremic pneumococcal pneumonia. Finally, one can show with a modification of the same technique the occurrence of circulating antigen-antibody complexes. An occasional patient with bacteremic pneumococcal pneumonia will have at the onset of infection a small but measurable quantity of antibody to the infecting pneumococcal type which declines steadily in amount during the early days of illness at which time no free antigen is detectable. If the patient's serum is treated with a proteolytic enzyme and reassayed for capsular antigen, the latter can then be identified.

As shown by Frisch et al. [9] in the 1940's, the amount of pneumococcal capsular polysaccharide present in the consolidated lung of the patient with lobar pneumonia may exceed a gram in amount. Although the preponderance is doubtless expectorated during recovery, it is not surprising that capsular polysaccharide should be detectable in the urine for a month or more after recovery.

Paul B. Beeson, who succeeded Blake as Chairman of the Department of Medicine in 1952, a post which he held until departing for Oxford in 1965 to become the Nuffield Professor of Medicine [10], was occupied for a time with some immunological aspects of pneumococcal infection in collaboration with Colin M. MacLeod, Charles L. Hoagland, and Walther F. Goebel during his tenure at the Rockefeller Institute. His initial publication concerned use of the Francis test, the intradermal injection of pneumococcal capsular polysaccharide, to monitor the adequacy of serum therapy of lobar pneumonia with either horse or rabbit antisera and highlighted several factors of critical importance to its proper interpretation [11]. In a similar vein, the utility of intravenous calcium chloride in arresting chills induced by antipneumococcal sera in treating pneumonia was reported [12].

Beeson's major contribution concerned the immunological relationship between the capsular polysaccharide of pneumococcus type 14 and the blood group A substance [13]. Earlier, Finland, and Curnan had reported from Boston on the severe reactions, hemoglobinuria, and occasional deaths following the administration of type 14 antipneumococcal horse, but not rabbit, antiserum. With Goebel, Beeson had observed also that human erythrocytes of all four blood groups were agglutinated to high titre by type 14 antipneumococcal horse sera, that absorption of the sera with human erythrocytes removed the agglutinins as did absorption with type 14 capsular polysaccharide but that absorption with the former did not significantly alter the titre of agglutinins of type 14 pneumococci. The phenomena were associated uniquely with equine antisera to this pneumococcal type.

Purified preparations of the capsular polysaccharide of pneumococcus type 14 were made and shown to consist of acetylglucosamine and galactose, major constituents of blood group A substance. Absorption of type 14 antipneumococcal horse serum with blood Group A substance removed approximately half the antibody nitrogen precipitable by type 14 polysaccharide [14].

The therapeutic problem posed by the use of type 14 antipneumococcal horse serum could be obviated by the use of antiserum prepared in rabbits in its stead. Differences in the specificities of antibodies of these two species were demonstrated further by Beeson and Goebel in a study of the cross reactivity of their respective antibodies to pneumococcus type 2 with Type B Friedländer bacillus, classified today as *Klebsiella pneumoniae* type 2 [15]. It had been reported earlier by Avery, Heidelberger, and Goebel that a cross reaction could be demonstrated between the capsular antigens of these two organisms. When the serologic reactions of rabbit and equine antibodies to pneumococcus type 2 with Type B Friedländer bacillus were examined, it was found that rabbit antibodies, unlike those from the horse, failed to give a Quellung reaction with Type B Friedländer bacillus, to precipitate its capsular polysaccharide or to protect mice against lethal infection. These observations were congruent with the earlier ones demonstrating the greater specificity of rabbit type 14 antipneumococcal serum, which, unlike horse antiserum to the same pneumococcal type, failed to cross-react significantly with human blood group substances.

A brief revival of interest in these observations occurred about a decade ago after the relicensure of polyvalent pneumococcal vaccine which included for the first time the capsular antigen of pneumococcus type 14 [16]. When it was discovered that some recipients of the vaccine were experiencing a rise in their blood group A isoagglutinins, the finding

was thought initially by some to be attributable to the type 14 capsular polysaccharide in the vaccine. It was shown subsequently, however, that the serologic findings were associated with vaccine prepared from pneumococci grown in a meat-based medium and that the problem could be obviated by substituting a medium of plant-based origin. These findings are consistent also with the fact that type 14 pneumococcal infection in man is not associated with hemolytic phenomena.

Studies of the bacteriologic properties of pneumococci were carried out by several investigators whose careers found them at one time or another on the faculty at Yale. One of them was John Rodman Paul [17]. Paul, who spent the greater part of his career at Yale, had encountered at an earlier time several men who were later to be his colleagues. As a child in Philadelphia, he had played with Stanhope Bayne-Jones, who was subsequently to instruct him in bacteriology at the Johns Hopkins University School of Medicine and "to demonstrate the recent discovery that the family of pneumococci could be divided into 'three' types on the basis of agglutinin reactions," all this many years before Bayne-Jones became Dean of Yale University School of Medicine [18]. Milton Winternitz, of whom more anon, "taught (pathology) by the method of terrorism: 'no knowledge without tears.' " Dr. Paul was to remark wryly at a later time that today it would "almost seem that it is the professor who experiences terrorism." Paul's medical education was interrupted by service with the Hopkins' base hospital in France during World War I, and he received his medical degree in 1919. After two years of internship at the Pennsylvania Hospital, he was named director of the Ayer Clinical Laboratory at that institution.

In the 1920s and 1930s, there was considerable interest in colonial variation of bacterial species infecting man. Noncapsulated pneumococci bad been isolated by Laura Stryker of the Rockefeller Institute in the preceding decade, and the attributes of the cells and colonies of so-called smooth virulent capsulated and rough avirulent noncapsulated variants of this species had been the object of considerable scrutiny. Paul published four papers on this topic, two in 1927 from Philadelphia [19–20] and two in 1934 subsequent to his coming to Yale [21–22]. Others of the Yale faculty who investigated variation in pneumococcal colonial morphology included Blake and James D. Trask [23], and Monroe D. Eaton [24–25], the last known best for his later work on mycoplasmas.

At that time, "smoothness" of bacterial colonies was associated with virulence in a number of species and "roughness" with the lack thereof. The use of these terms sometimes took a bizarre twist, as in the case of *Bacillus anthracis*, of which rough colonial forms were referred to as "S" variants because of their attribute of virulence. The problem was one with which I became involved somewhat later. In an attempt to obtain noncapsulated variants of pneumococci without having to make and employ anticapsular serum, a possibility suggested by some reported studies of group B streptococci [26], capsulated pneumococci were grown as widely separated colonies on sealed blood agar plates incubated for 10 to 14 days [27]. Excrescences at the margins of such colonies were distinctly rough in appearance and were thought initially to represent noncapsulated variants. A Quellung test with homotypic anticapsular serum, however, showed these so-called "rough" colonial variants to be composed of fully capsulated cells growing in long chains or filaments. If the inoculum employed in the initial experiment was replaced by a noncapsulated diplococcal variant, the colonial excrescence in this instance would be composed of noncapsulated cells growing in long chains or filaments. From these experiments, it would seem reasonable to conclude that it is more informative to look at bacterial cellular attributes than colonial ones. Studies of phagocytosis *in vitro* and of mouse virulence show clearly the survival value of the nonfilamentous variants over that of the filamentous ones.

Paul sought, both at the Ayer Clinical Laboratory [20] and at Yale [22], to determine if R or noncapsulated pneumococci could be identified as a component of the human upper respiratory flora. The taxonomic separation of pneumococci from other alpha hemolytic streptococci is a difficult one though it may be nearing resolution. The overall base pair composition of the DNA of pneumococci and some alpha streptococci is very similar, being composed of 38 mol percent guanine plus cytosine. DNA-mediated interspecific transformation between organisms of the two groups may be carried out with either serving as donor or recipient of streptomycin resistance. Many non-pneumococcal streptococci are capsulated, and their capsular polysaccharides may be very similar to those of some pneumococcal types. Antibodies to these alpha streptococci may be protective against infection with cross-reactive pneumococcal serotypes and may play a role in so-called "natural" immunity [28]. Noncapsulated pneumococci instilled into the human nose can be recovered from the upper respiratory tract as long as two weeks after their placement there, but it remains an unanswered question whether or not such variants ever comprise a component of the normal human upper respiratory flora.

Two notable members of Yale's medical faculty carried out extensive studies of the pathogenesis of pneumococcal pneumonia: Milton C. Winternitz, who became Dean of the School of Medicine in 1920 and is credited with its renaissance, and Francis G. Blake, the other investigator of this problem, chosen by Winternitz to be chairman of the Department of Medicine.

Winternitz was born in Baltimore in 1885 and attended medical school at Johns Hopkins at the same time as my father. A colorful account of his role as a teacher of pathology is given by John R. Paul in volume 43 of *The Yale Journal of Biology and Medicine* [29]. Winternitz published a series of nine papers with several collaborators on the pathogenesis of pneumococcal pneumonia in *The Journal of Experimental Medicine* between 1913 and 1915, which, together, provide ample evidence of his ability as an experimentalist [30–38]. These studies, in which pneumonia was induced by inoculation through an intratracheal catheter of an untyped pneumococcus obtained from the blood of a human patient, were carried out both in normal rabbits and in animals rendered agranulocytic with benzol. They give insights into the susceptibility of the mammalian lung to infection, the role of polymorphonuclear leukocytes in defense against infection and the failure of trypanocidal dyes to affect the pneumococcus. Among the more ingenious and intriguing of these investigations are the studies of *intra vitam* staining of the lung and its vasculature in experimental pneumonia [37]. When one percent trypan blue was injected intravenously into a rabbit prior to pneumococcal infection of the lung, the pneumonic area was stained uniformly, especially the fibrinous exudate. Many leukocytes failed to take up the dye, however, indicative of the fact that they were still viable. If, however, injection of the dye was delayed until 20 to 65 hrs after infection of the lung, pale consolidated areas were present in the involved lobes resembling wedge-shaped infarcts. The prevailing view at the time was that pallor of the affected lung was the result of capillary compression by the alveolar exudate. To test this hypothesis, pneumonic lungs of both humans and rabbits were injected through the pulmonary artery with a Berlin blue gelatin solution under a pressure of 120 mm Hg, the pulmonary artery tied and the lungs fixed. On sectioning, the consolidated areas were pale gray, the unaffected areas of the lung intensely blue; and, on microscopic examination, the consolidated areas were remarkable for the absence of dye from the capillaries. When one lung of a normal pair was hyperinflated and the pulmonary artery injected with dye as before, there was no difference in the amount of dye in the alveolar capillaries of the two lungs. Even when one lung was filled through the bronchus with dye solution at a pressure of 120 mm Hg, there was no difference in the vessels of the two lungs after perfusion of the pulmonary artery. Neither did

rendering the animals leukopenic with benzol alter the distribution of intravenously injected dye. In the leukopenic rabbit, the exudate consisted almost entirely of fibrin. To inhibit the formation of fibrin, animals were injected subcutaneously with an old solution of phosphorus in olive oil and given a small amount of chloroform in 30% alcohol intragastrically. In 24 hrs, clotting was observed to be delayed and the clot jelly-like. Such an animal inoculated intrabronchially with a pneumococcal culture died in 17 1/2 hrs with consolidation of the left lower lobe. The lungs were injected through the pulmonary artery as before, the lungs fixed and sectioned. The consolidated area on this occasion was uniformly blue and its vessels contained dye. The fibrin plugs found in areas of consolidation of normal animals were absent. It was hypothesized by the investigators in these ingenious experiments that the absence of antitryptic substance in serum secondary to impairment of capillary circulation in the normal animal favored resolution of the pneumonic lesion by the uninhibited action of leukocytic enzymes in the pneumonic exudate. Their observations are consistent also with the now accepted concept that cyanosis in pneumonia is not the result of blood circulating through unaerated lung, but rather the consequence of impaired alveolar ventilation secondary to rapid shallow breathing.

Five years later, a second series of investigations of experimental pneumococcal pneumonia, this time in primates, was reported by Francis G. Blake and Russel L. Cecil in *The Journal of Experimental Medicine* [39–45]. The investigations, carried out at the Bacteriological Laboratory of the Army Medical School in Washington, were prompted by the military experience with influenza and pneumonia during the pandemic of 1918–1919. Disease was produced in Philippine macaques and Central American Cebus monkeys by injecting an inoculum of pneumococci into the trachea through a needle inserted between the tracheal cartilages. In their initial report, Blake and Cecil concluded that the pneumococcus is the specific cause of lobar pneumonia, that neither colonization of the upper respiratory tract nor intravenous injection of pneumococci gave rise to pneumonia but that pneumonia is bronchogenic in origin and that bacteremia is secondary to invasion of the lungs.

Blake and Cecil's observations on the pathology and pathogenesis of pneumococcal pneumonia, however, seem somewhat at variance with contemporary views on these subjects [40]. It was their conclusion that the pneumococcus invades the tissue of the lung at some point near the root of the affected lobe and spreads peripherally *via* the interstitium and lymphatics of the organ; i.e., that lobar pneumonia is primarily an interstitial infection. Winternitz was aware of these studies from oral presentations of their findings by Blake and Cecil before several societies in 1919. In a report published in 1920 in the *Bulletin* of the Johns Hopkins Hospital entitled "An Unrecognized Pathway for Bacterial Invasion of the Lower Respiratory Tract," Winternitz described the extensive plexus of lymphatic vessels underlying the tracheal mucosa which drained downward to the hilum of the lung to involve the bronchial, pleural, and mediastinal lymphatics [46]. The withdrawal of an infected needle through the tracheal wall gave rise to a descending infection in the rabbit with striking resemblance to the findings of Blake and Cecil. Infection of the lung through a tracheobronchial catheter, on the other hand, successfully introduced to avoid mucosal trauma, gave rise to a pneumonitis characterized by spread through the airways as is accepted generally today. The story is a dramatic example of how an experimental detail can alter the outcome of an investigation.

The collaborators at the Army Medical School went on to describe spontaneous pneumococcal pneumonia in monkeys [41], the protective effect of recovery from sublethal infection against reinfection with the same pneumococcal type [44], and the therapeutic effect of passively administered type specific antipneumococcal serum prepared by the New York State Board of Health, presumably in horses [45].

Dr. Blake maintained a lifelong interest in pneumococcal pneumonia and its treatment and published on each of the therapeutic advances as sulfonamides largely replaced antiserum, and penicillin, in turn, replaced sulfonamides [47]. One of the therapeutic approaches explored in the 1930's and now largely forgotten, was the use of pneumothorax. Between 1934 and 1940. Blake published six articles on the subject including a lengthy review in the journal *Medicine* [48]. The investigation was inspired by the publication of Lt. Rood, Medical Corps, U.S. Army, in 1919 of the results of pneumothorax occurring as a sequel to diagnostic lung puncture: "It was interesting to note that often the patient was relieved by lung puncture, especially if pneumothorax followed the operation, and it was not uncommon for patients to request that the operation be repeated. Patients frequently showed a drop in temperature following puncture and a temporary improvement in physical signs. The cough and sputum would increase for a short time, followed by a marked decrease in the number of râles within a few hours." After extensive investigation, Blake concluded in 1938: "Artificial pneumothorax would appear to be of value for the relief of pleural pain in selected early cases of lobar pneumonia. It has not been demonstrated that it possesses any curative value in this disease" [49]. The introduction of penicillin for the treatment of pneumococcal pneumonia in 1944 was to make all such therapies obsolete.

Cecil and Blake were stimulated by the epidemics of respiratory disease in 1918 and 1919 to investigate also the efficacy of prophylactic vaccination against pneumococcal pneumonia in the monkey. Large scale trials of vaccines of whole killed pneumococcal cells in man had been initiated in South Africa in 1911 by Sir Almroth Wright and continued by his protégé, F. Spencer Lister, with inconclusive results [50]. Similar trials of two trivalent vaccines were conducted in this country by Cecil in 1918, one at Camp Upton in New York in collaboration with J. Harold Austin [51], who like a former member of the Yale medical faculty, William Stadie, was an illustrious predecessor of mine in the Department of Research Medicine at the University of Pennsylvania. The other trial was carried out at Camp Wheeler in Georgia [52]. Both trials, each including more than 10,000 recipients of vaccine, were suggestive of the prophylactic value of vaccination in reducing the incidence of pneumococcal pneumonia. Careful analysis of the data, however, failed to establish unequivocally such a conclusion. To study further the question of active immunity, Cecil and Blake undertook, therefore, a series of investigations in monkeys.

For their source of vaccine, Cecil and Blake chose the type 1 strain of pneumococcus included in the military vaccines, one of greatly reduced virulence that might well be classified today as an intermediate capsular variant producing small amounts of capsular polysaccharide [42]. Variants of this kind, as has been learned subsequently, may arise from mutations in any of the several genes in the multigenic pathway controlling the synthesis of the pneumococcal capsule [53]. As had been the case in the two military trials, two types of vaccine, one a saline suspension of heat-killed organisms, the other a "lipovaccine" consisting of the ground sediment of heat-killed organisms suspended in cottonseed oil, were employed, Although the number of pneumococcal organisms injected in each of the several experiments is known, the amount of type 1 capsular polysaccharide cannot be calculated and equated with that in other vaccines.

Monkeys receiving either vaccine were challenged by intratracheal inoculation with a highly virulent type 1 pneumococcal strain or by exposure to a cage mate suffering from type 1 pneumococcal pneumonia. Neither vaccine prevented infection in the inoculated animals although the degree of bacteremia in the vaccinated animals was less than

that in their unvaccinated counterparts. The reasons for the failure of the vaccines may have been several. First, it is not clear than a sufficient amount of type 1 pneumococcal capsular polysaccharide had been injected to induce immunity and second, in those animals challenged by intratracheal inoculation, the challenge may have been excessive.

In addition to the studies with killed vaccines, the immunity resulting from subcutaneous vaccination with living cultures of pneumococci was explored [43]. Both a virulent and the previously studied avirulent type 1 pneumococcal strain were employed. Two of those animals surviving vaccination with the live virulent strain proved immune to challenge. Studies of the live aviruent pneumococcal vaccine were limited to two vaccinated animals and one control. The authors concluded that: "Living avirulent pneumococci, when injected subcutaneously, appear to excite an immunity equal in degree to that produced by living virulent pneumococci, if a large enough dose is given."

In all their studies of immunization with killed and with live pneumococcal vaccines, 24 animals were vaccinated and 14 served as controls. When the various protocols are taken also into account, the biostatistical probabilities of the results observed not having occurred by chance would almost certainly fail to meet contemporary criteria of acceptability. Although biostatistical analysis had been advocated in 1913 by Major Greenwood in his caustic assessment of Sir Almroth Wright's pneumococcal vaccine trials published in *Lancet* [54], the application of such methods had not yet achieved the wide use they enjoy today.

Three years after Blake and Cecil published their studies of pneumococcal infection in monkeys, Schiemann and Casper reported the antigenicity of a pneumococcal capsular polysaccharide in mice [55], and, 22 years after their report, MacLeod and his coworkers demonstrated, in an elegantly structured and controlled trial, that as little as 50 μg of a purified pneumococcal capsular polysaccharide injected into man could effectively prevent infection with the homotypic organism [56]. Space does not permit a recounting of subsequent events in the history of pneumococcal vaccines which are reviewed elsewhere. Suffice it to say that relicensure of such a vaccine in 1977 and release of an expanded formulation of the vaccine in 1983 have not been unattended by controversy [57]. One reason is the failure of some to take into account the inability of the aggregate efficacy of a vaccine designed to prevent 23 immunologically distinct infections to equal that of a monovalent vaccine, which clearly it cannot. Others arise from recommendations that the vaccine be administered to immunocompromised patients incapable of responding to the antigenic stimulus of the vaccine without stressing adequately the likelihood of its failure in some such patients. Studies at Yale by Eugene D. Shapiro, John D. Clemens, and their associates have been major contributions to a better understanding of the vaccine's role [58–59], one which may become of increasing importance as mounting numbers of pneumococci manifesting resistance to multiple antimicrobial drugs are isolated from infected humans. Unanswered questions remain concerning the need or lack thereof to revaccinate and the wisdom of delaying vaccination until the seventh decade of life as is now recommended. It is gratifying to know that interest in the pneumococcus persists at Yale. It bodes well for all.

REFERENCES

1. Austrian, R. The pneumococcus at Hopkins: early portents of future developments. Johns Hopkins Med. J. 144:192–201, 1979.
2. Austrian, R. The Gram stain and the etiology of lobar pneumonia. Bact. Rev. 24:261–265, 1960.
3. Austrian, R. The Quellung reaction, a neglected microbiologic technique. Mount Sinai J. Med. 43:699–709, 1976.
4. Paul, J. R. Francis Gilman Blake. 1887–1952. Yale J. Biol. Med. 24:436–449, 1952.
5. Blake, F. G. Methods for the determination of pneumococcus types. J. Exp. Med. 26:67–

80,1917.
6. Blake, F.G. Antigen-antibody balance in lobar pneumonia. Arch. Intern. Med. 26:779–790, 1917.
7. Schiffman, G., Doulgas, R. M., Bonner, M. J., Robbins, M., and Austrian, R. A radioimmunoassay for immunologic phenomena in pneumococcal disease and for the antibody response to pneumococcal vaccines. 1. Methods for the radioimmunoassay of anticapsular antibodies and comparison with other techniques. J. Immunol. Meth. 30:133–144, 1980.
8. Kaplan, M. H., Coons, A. H., and Deane, H. W. Localization of antigens in tissue cells. III. Cellular distribution of pneumococcal polysaccharides types II and III in the mouse. J. Exp. Med. 91:15–30, 1950.
9. Frisch, A. W., Tripp, J. T., Barrett, C. D., Jr., and Pidgeon, B. E. The specific polysaccharide content of pneumonic lungs. J. Exp. Med. 76:505–510, 1942.
10. Petersdorf, R. G. Presentation of the Kober Medal for 1973 to Paul B. Beeson. Trans. Assoc. Amer. Phys. 86:46–61, 1973.
11. MacLeod, C. M., Hoagland, C. L., and Beeson, P. B. The use of the skin test with type specific polysaccharides in the control of serum dosage in pneumococcal pneumonia. J. Clin. Invest.17:739–744, 1938.
12. Beeson, P. B. and Hoagland, C. L. Use of calcium chloride in relief of chills following serum administration. Proc. Soc. Exp. Biol. Med. 38:160–162, 1938.
13. Beeson, P. B. and Goebel, W. F. The immunological relationship of the capsular polysaccharide of type XIV pneumococcus to the blood group A specific substance. J. Exp. Med. 70:239– 247, 1939.
14. Goebel, W. F., Beeson, P. B., and Hoagland, C. L. Chemo-immunological studies on the soluble specific substance of pneumococcus. IV. The capsular polysaccharide of type XIV pneumococcus and its relationship to the blood group A specific substance. J. Biol. Chem. 129:455–464, 1939.
15. Beeson, P. B. and Goebel, W. F. Immunological cross-reactions of type B Friedländer bacillus in type 2 antipneumococcal horse and rabbit serum. J. Immunol. 38:231–235, 1940.
16. Siber, G. R., Ambrosino, D. M., and Gorgone, B. S. Blood-group-A-like substance in a preparation of pneumococcal vaccine. Ann. Intern. Med. 96:580–586, 1982.
17. Horstmann, D. M. and Beeson, P. B. John Rodman Paul. April 18,1893–May 6,1971. Biographical Memoirs, Natl. Acad. Sci., U.S.A. 47:323–368,1975.
18. Paul, J. R. Stanhope Bayne-Jones (1888–1970). Medical dean, military hero, microbiologist and epidemiologist, and medical historian. Yale J. Biol. Med. 45:22–32, 1972.
19. Paul, J. R. A comparative study of smooth and rough pneumococcus colonies. J. Exp. Med. 46:793–805, 1927.
20. Paul, J. R. The occurrence of rough pneumococci *in vivo*. J. Exp. Med. 46:807–817, 1927.
21. Paul, J. R. Pneumococcus variants. I. Intermediate forms and the influence of environment in their production during *in-vitro* S to R and R to S transitions. J. Bact. 28:45–66, 1934.
22. Paul, J. R. Pneumococcus variants. II. Dissociants of R pneumococci and their relation to Streptococcus viridans. J. Bact. 28:69–92, 1934.
23. Blake, F. G. and Trask, J. D. Pneumococcus variants intermediate between the S and R forms. J. Bact. 25:289–307, 1933.
24. Eaton, M. D. Studies on pneumococcus variation. I. Variants characterized by rapid lysis and absence of normal growth under the routine method of cultivation. J. Bact. 27:271–291, 1934.
25. Eaton, M. D. Studies on pneumococcus variation. II. Smooth virulent variants produced by daughter-colony dissociation of smooth pneumococcus strains. J. Bact. 30:119–134, 1935.
26. Pomales-Lebrón, A. and Morales-Otero, P. Dissociation among Lancefield's Group B streptococci of human and bovine origins. Proc. Soc. Exp. Biol. Med. 70:612–616, 1949.
27. Austrian, R. Morphologic variation in pneumococcus. I. An analysis of the bases for morphologic variation in pneumococcus and description of a hitherto undefined morphologic variant. J. Exp. Med. 98:21–34, 1953.
28. Yurchak, A. M. and Austrian, R. Serologic and genetic relationships between pneumococci and other respiratory streptococci. Trans. Assoc. Amer. Phys. 79:368–375, 1966.
29. Paul, J. R. Dean Winternitz and the rebirth of the Yale Medical School in the 1920's. Yale J. Biol. Med. 43:110–119, 1970.
30. Winternitz, M. C. and Hirschfelder, A. D. Studies upon experimental pneumonia in rabbits. I. The production of lobar pneumonia. J. Exp. Med. 17:657–660, 1913.
31. Winternitz, M. C. and Hirschfelder, A. D. Studies upon experimental pneumonia in rabbits. II. Pneumonia in animals rendered aplastic. J. Exp. Med. 17:660–662, 1913.
32. Winternitz, M. C. and Hirschfelder, A. D. Studies upon experimental pneumonia in rabbits. III.

Intra vitam staining. J. Exp. Med. 17:662–665,1913.
33. Hirschfelder, A. D. and Winternitz, M. C. Studies upon experimental pneumonia in rabbits. IV. Is there a parallelism between the trypanocidal and pneumococcidal action of drugs? J. Exp. Med. 17:666–678, 1913.
34. Kline, B. S. and Winternitz, M. C. Studies upon experimental pneumonia in rabbits. V. The role of the leukocyte in experimental pneumonia. The relation of the number of organisms injected to the mortality. J. Exp. Med. 18:50–60, 1913.
35. Kline, B. S. and Winternitz, M. C. Studies upon experimental pneumonia in rabbits. VI. Studies in immunity. J. Exp. Med. 18:61–74, 1913.
36. Kline, B. S. and Winternitz, M. C. Studies upon experimental pneumonia in rabbits. VII. The production of lobar pneumonia. J. Exp. Med. 21:304–310, 1915.
37. Kline, B. S. and Winternitz, M. C. Studies upon experimental pneumonia in rabbits. VIII. *Intra vitam* staining in experimental pneumonia and the circulation of the lung. J. Exp. Med. 21:311–319, 1915.
38. Winternitz, M. C. and Kline, B. S. Studies upon experimental pneumonia in rabbits. IX. The part of the leukocyte in immunity reactions. J. Exp. Med. 21:320–329, 1915.
39. Blake, F. G. and Cecil, R. L. Studies on experimental pneumonia. I. Production of pneumococcus lobar pneumonia in monkeys. J. Exp. Med. 31:403–443, 1920.
40. Blake, F. G. and Cecil, R. L. Studies on experimental pneumonia. II. Pathology and pathogenesis of pneumococcus lobar pneumonia in monkeys. J. Exp. Med. 31.445–474, 1920.
41. Blake, F. G. and Cecil, R. L. Studies on experimental pneumonia, III. Spontaneous pneumonia in monkeys. J. Exp. Med. 31:499–518, 1920.
42. Cecil, R. L. and Blake, F. G. Studies on experimental pneumonia. IV. Results of prophylactic vaccination against pneumococcus pneumonia in monkeys. J. Exp. Med. 31:519–553, 1920.
43. Cecil, R. L. and Blake, F. G. Studies on experimental pneumonia. V. Active immunity against experimental pneumococcus pneumonia in monkeys following vaccination with living cultures of pneumococcus. J. Exp. Med. 31:657–683, 1920.
44. Cecil, R. L. and Blake, F. G. Studies on experimental pneumonia. VI. Active immunity following experimental pneumococcus pneumonia in monkeys. J. Exp. Med. 31:685–708, 1920.
45. Cecil, R. L. and Blake, F. G. Studies on experimental pneumonia. VII. Treatment of experimental pneumococcus type I pneumonia with type I antipneumococcus serum. J. Exp. Med. 32:1–18, 1920.
46. Winternitz, M. C., Smith, G. H., and Robinson, E. S. An unrecognized pathway for bacterial invasion of the lower respiratory tract. Bull. Johns Hopkins Hosp. 31:63–66, 1920.
47. Bibliography of Francis G. Blake. Yale J. Biol. Med. 24:444–449, 1952.
48. Blake, F. G., Howard, M. E., and Hull, W. S. Artificial pneumothorax in lobar pneumonia. Medicine 15:1–101, 1936.
49. Blake, F. G. A further evaluation of artificial pneumothorax in lobar pneumonia. J. Am. Med. Assn. 111:581–584, 1938.
50. Austrian, R. Of gold and pneumococci. A history of pneumococcal vaccines in South Africa. Trans. Amer. Clin. Climat. Assoc. 39:141–161, 1977.
51. Cecil, R. L. and Austin, J. H. Results of prophylactic inoculation against pneumococcus in 12,519 men. J. Exp. Med. 28:19–41, 1918.
52. Cecil, R. L. and Vaughan, H. F. Results of prophylactic vaccination against pneumonia at Camp Wheeler. J. Exp. Med. 29:457–483, 1919.
53. Austrian, R., Bernheimer, H. P., Smith, E. E. B., and Mills, G. T. Simultaneous production of two capsular polysaccharides by pneumococcus. II. The genetic and biochemical bases of binary capsulation. J. Exp. Med. 110:585–602, 1959.
54. Greenwood, M. On methods of research available in the study of medical problems with special reference to Sir Almroth Wright's recent utterances. Lancet 1:158–165 ,1913.
55. Schiemann, O. and Casper, W. Sind die spezifisch präcipitabeln Substanzen der 3 Pneumokokkentypen Haptene? Zeits. Hyg. Infektionskr. 108:220–257, 1927.
56. MacLeod, C. M., Hodges, R. G., Heidelberger, M., and Bernhard, W. G. Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. J. Exp. Med. 82:445–465, 1945.
57. Clemens, J. D. and Shapiro, E. D. Resolving the pneumococcal vaccine controversy: are there alternatives to randomized clinical trials? Rev. Infect. Dis. 6:589–600, 1984.
58. Shapiro, E. D. and Clemens, J. D. A controlled evaluation of the protective efficacy of pneumococcal vaccine for patients at high risk of serious pneumococcal infection. Ann. Intern. Med. 101:325–330, 1984.
59. Shapiro, E. D., Berg, A. T., Austrian, R., Schroeder, D., Parcells, V., Margolis, A., Adair, R. K., and Clemens, J. D. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. N. Eng. J. Med. 325:1453–1460, 1991.