

THE VIABILITY OF THE PNEUMOCOCCUS AFTER DRY-
ING: A STUDY OF ONE OF THE FACTORS
IN PNEUMONIC INFECTION.

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The exact way in which the pneumococcus reaches the lungs of persons suffering from pneumonia due to that organism is not yet thoroughly understood. A number of possibilities have been considered which may be indicated as follows:

(1) The first is that the pneumococcus is frequently present in the saliva, and that when the resistance of a person carrying these organisms is reduced, for example by exposure or overwork, an infection of the lungs takes place either by extension along the tracheal mucosa or by the direct aspiration into the lung of particles of the salivary secretion carrying the germs with them.¹

(2) A second possibility which may be considered is that the pneumococcus is transferred from the oral or pharyngeal mucosa to the lungs by the lymphatics or through the blood.

(3) Another possibility is that the pneumococcus, which is capable of living in masses of dry sputum for some time, is distributed in the form of dust derived from the dried sputum particles and that these particles are inhaled, thus giving rise to a pulmonary infection.

(4) A fourth suggestion is that the pneumococcus is carried

¹ For phases of this problem which cannot be considered here, see Wadsworth, *American Jour. of the Med. Sciences*, 1904, cxxvii, 851. Other papers on the subject are: Nenninger, *Zeit. f. Hyg.*, 1901, xxxviii, 94; Klipstein, *Zeit. f. klin. Med.*, 1898, xxxiv, 191; Dürck, *Deutsches Arch. f. klin. Med.*, 1897, lviii, 368; Wandel, *ibid.*, 1903, lxxviii, 1.

directly from person to person either by the transfer of the normal nasal or salivary fluids, which may contain pneumococci, by coughing or sneezing, or by the spraying of fine particles derived from the sputum of those suffering from pneumonia or other acute inflammations of the air passages, by the same mechanical processes, and that the spray particles thus formed carry virulent organisms to the lungs.

The present study is devoted to a consideration of the possibilities of the aërial transmission of the pneumococcus either in the form of sprayed particles or as dust derived from dried sputum, the modes of infection from the saliva as given in the first and second paragraphs not coming within the scope of this investigation.

While a good deal of work has been done by Cornet, Flügge, and others in determining the viability of the tubercle bacillus and other organisms in fine spray and also after drying and subjection to various physical agents, but little attention has been directed to the pneumococcus except when dried in relatively large masses of sputum. Most observers have considered the pneumococcus as an organism incapable of living for any considerable time when suspended in the form of fine spray.

As the results of recent studies² on the biology of the pneumococcus have rendered the identification of that organism relatively easy, and as some of the earlier studies on the viability were carried out with bacteria which may or may not have been the pneumococcus, it seemed to the writer that a revision and extension of some of the older investigations might be of value in deciding some points in the mode of transmission of this organism which, though important, have not yet been cleared up.

HISTORICAL RÉSUMÉ.

Before proceeding to the description of the methods and results of personal experiments, it may be well to give a short résumé of the work done by other observers on the general question of transmissibility of the pneumococcus from infectious material to human beings.

² Hiss, *Jour. of Exper. Med.*, 1905 vi, 317.

Viability of Pneumococci in Dried Sputum.—The earlier experiments to determine the dangers of air infection by the pneumococcus were conducted with the idea of fixing the length of time during which the organism would remain virulent for rabbits or mice after drying sputum in bulk, the powdering and diffusion of the powder by air currents being thought to be the means of transmission.

It was known that the pneumococcus died very rapidly in many of the ordinary culture media. In fact it was pointed out by Kruse and Pansini³ that some varieties of bouillon made from meat infusion were highly bactericidal to the pneumococcus. The same observers found that in body fluids, however, for example in sealed tubes containing pleuritic exudate, the pneumococcus may remain alive for more than a year, if kept in a cool, dark place. They showed that in moist sputum preserved at 15° C. the life of the organism is very short, usually but three or four days. In sputum kept within a few degrees of 0° C., however, the life of the organism is much longer, and while the fluid loses in a few days much of its virulence for mice, yet living pneumococci can be demonstrated for at least six weeks under these conditions.

Drying of the sputum in the air at incubator temperatures killed the organisms quickly, though Guarnieri⁴ found that rapid drying in a desiccator at 37° C. preserved the virulence for rabbits for four months.

Patella⁵ noted that rapid drying over sulphuric acid at 16° C. or 38° C. killed the organisms promptly, while slow drying at low temperatures enabled them to live for some time. As it has been shown by Kirstein⁶ that sulphuric acid probably gives off a small quantity of sulphur trioxide, which would act destructively upon any organisms with which it might come into contact, the rapid death frequently observed when the pneumococcus is desiccated over this medium may be due to the bactericidal

³ *Zeit. f. Hyg.*, 1892, xi, 279.

⁴ *Atti della R. Accad. med. di Roma*, 1888, iv, 97.

⁵ *Ibid.*, 447.

⁶ *Zeit. f. Hyg.*, 1902, xxxix, 166.

action of the acid. Drying over calcium chloride and phosphoric anhydride does not destroy bacteria so quickly as drying over sulphuric acid.

Foà and Bordoni-Uffreduzzi⁷ dried rabbit's blood containing pneumococci on watch glasses and found that the micro-organisms were alive and virulent after forty-five days. Agar tubes inoculated from organs and kept for sixty days showed an abundant growth when placed in the incubator—an evidence that the organisms may remain alive under suitable conditions. At the time at which this work was done, however, the difference between the pneumococcus and the meningococcus had not been thoroughly defined, and the writers termed the organism with which they worked a meningococcus because it was obtained from a case of cerebrospinal meningitis. From their description of its biological features, however, the organism seems to have been *Diplococcus pneumoniae*. It was positive to Gram, showed a capsule, and killed rabbits promptly. Apparently without recognizing their nature, the writers figure pneumococci inclosed in the phagocytic cells of the pneumonic exudate of the rabbit.

Five years later Bordoni-Uffreduzzi⁸ reported the results obtained by contaminating pieces of linen with pneumonic sputum. The cloth was allowed to dry at room temperatures. In one specimen exposed to diffuse light the bacteria remained alive for nineteen days, as determined by injection into rabbits of scrapings from the cloth; in another, fifty-five days. The sputum dried in sunlight was virulent after twelve hours. The results differ somewhat from those of Patella, possibly depending upon the technique, Patella using silk threads soaked in the blood of an animal dying of a pneumococcus septicæmia or threads soaked in broth cultures of the pneumococcus.

The results of Cassedebat⁹ differ considerably from those of Bordoni-Uffreduzzi. The experiments were conducted as follows: Sputum was tested for its virulence and found to kill rabbits. Specimens of this pneumonic sputum were then dried

⁷ *Zeit. f. Hyg.*, 1888, iv, 67.

⁸ *Arch. p. l. sc. med.*, 1891, xv, 341.

⁹ *Revue d'Hygiène*, 1895, xvii, 1066.

on cloth in the air but protected from the direct rays of the sun. Fragments of the cloth were soaked in water and the fluid injected into a rabbit. The results showed that the dried sputum killed rabbits at periods varying from five to twenty-six days, and that fresh sputum from the eighth and ninth days of the disease would not kill rabbits. Apparently the writer relied on the gross post-mortem findings for the identification of the pneumococcus. There is no mention of morphological studies of the blood to identify capsulated organisms or attempts to cultivate the pneumococci. The results have, therefore, but slight value.

Ottolenghi¹⁰ repeated the studies of Bordoni-Uffreduzzi with the following results. The experiments were carried out with three specimens of pneumonic sputum from the fourth or fifth day of the disease. The sputum was spread on linen cloth and allowed to dry in diffuse light at a temperature of 15° C. to 20° C. In explaining the results which he obtained, the author calls attention to the fact that the inoculation of the material into a rabbit is not sufficient to determine whether the pneumococcus is dead or not. The death of the rabbit merely determines the presence of organisms virulent for that animal, but non-virulent forms may be present. He therefore made cultures from the sputum at the same time that he carried out the animal inoculations. The first specimen tested lost its virulence for animals thirty-six days after the preparation was made, whereas pneumococci could be obtained culturally for sixty days after drying. In the second specimen, both methods showed pneumococci at the end of seventy days, and from the third specimen pneumococci were isolated on the eighty-third day. On the basis of these experiments, the author considered that *Diplococcus lanceolatus* can retain its virulence in dried sputum for at least twenty days, and that it remains alive for a considerable time after the virulence has disappeared. The virulence persisted longest in a thin, frothy sputum.

In some experiments recently reported by Heim,¹¹ the viability

¹⁰ *Cent. f. Bakt.*, 1899, xxv, Abt i, 120.

¹¹ *Zeit. f. Hyg.*, 1905, l, 123.

of the pneumococcus after drying was much greater than has usually been assumed. Silk threads were dipped into the heart's blood of cats, rabbits, and mice, which had been killed by injections of pneumococci. The threads were dried in a desiccator over calcium chloride, and were then removed at various periods, placed in bouillon or agar, and the resulting culture inoculated into mice. The organisms were virulent in some cases after 487 days. Great variations, however, were observed. Some of the cultures from the threads no longer gave rise to septicæmia after sixty-six days. One case was not virulent after nine days. In empyema pus the pneumococcus remained virulent for 377 days. Another specimen of empyema pus contained organisms which Heim states lie between the pneumococcus and the streptococcus groups. These were virulent at the end of 149 days and contained viable organisms at the end of 383 days. The conditions of these experiments are, however, highly artificial and cannot be considered as applying very definitely to the question of aerial infections. The alternate drying and moistening of the organisms due to the varying amounts of moisture in the air of rooms is very important as determining the rapid death of the pneumococcus, while protection from such changes by sealing the substances carrying the bacteria in vessels containing calcium chloride tends to prolong the life of the parasite.

Mode of Distribution of Dried Sputum Particles.—It thus having been conclusively shown that the pneumococcus can remain alive for a considerable length of time in dried sputum, it is necessary to demonstrate that this dried sputum, which under ordinary conditions is firmly adherent to the substance on which it is dried, can in some way be reduced to a powder and thus inhaled. Such conditions can only be realized when the sputum is dried in handkerchiefs, bedding, or clothing, and the contaminated material handled, or when the sputum is deposited upon the floor and pulverized by persons walking over the infected area, or distributed in the air by dry sweeping of the floor, or brushing of infected clothes, etc. This mode of distribution of infectious material has been studied chiefly in connection with the tubercle bacillus, because of the ease of identifying that organism in the

infectious dust, and the difficulty attendant upon the recognition of the pneumococcus under the same conditions. The results obtained, however, can be legitimately transferred to the pneumococcus, leaving out of the question for the moment the viability of the latter organism after drying. Much of our knowledge on this subject we owe to the studies of Cornet and of Flügge and his pupils.

Some of the results which have been obtained are as follows: Cornet,¹² who considers this dust inhalation the most important means of infection in tuberculosis, has demonstrated the infectious nature of the dust of rooms in which persons suffering from tuberculosis had lived, and showed that the risk of infection depended very largely upon the expectoration of the sputum on handkerchiefs, bedding, carpets, or clothing, and the subsequent drying of the fluid. He considered that there was practically no danger of direct infection in tuberculosis by particles of sputum expelled by coughing, but that the sputum expectorated in large masses and dried on the bedding or floor was the chief source of the disease. His results have been disputed by Fränkel and also by Flügge and his pupils, who have shown that it is difficult to pulverize the sputum to a sufficient degree to produce a powder fine enough to be carried by air currents of moderate velocity or to remain long in suspension.

Sticher,¹³ in order to test this, pulverized dried tuberculous sputum and found that while the particles could be carried by a current of air with a velocity of 1 cm. per second to a height of one meter, yet the number of bacteria which even the very fine dust particles carried was small, and hence infection was not likely to result from the dissemination of such dust. The air currents in rooms without special ventilation rarely exceed one centimeter per second and do not transport for any length of time coarse dust such as is produced by powdering sputum.

Beninde¹⁴ repeated the experiments of Sticher and found that

¹² *Die Tuberculose, Nothnagel's Spec. Path. u. Therap.*, Bd. xiv, p. 209. Vienna, 1899.

¹³ *Zeit. f. Hyg.*, 1899, xxx, 163.

¹⁴ *Ibid.*, 193.

using handkerchiefs contaminated with tuberculous sputum it was impossible while the latter was still damp to remove any bacilli from the surface of the cloth by a stream of air with a velocity of 10 cm. per second, this being the upper limit of air currents in well ventilated rooms. Tubercle bacilli, however, could be removed by using an air stream of 1 cm. per second, but only after the handkerchief had been carried about for two days and was thoroughly dry.

Further investigations in this line were made by Neisser,¹⁵ who used pneumonic sputum and studied the conditions obtained by mixing the fluid with dust and drying. This combination was then finely pulverized and carried from one chamber to another by an air current with a speed of from 2.8 mm. per second to 23 cm. per second. As a control, some of the sputum used was injected into mice and shown to be virulent. As soon as pneumonic sputum and the dust mixture dried, it would no longer kill mice, therefore the danger of dust infection by inhaling pulverized and dried sputum seemed exceedingly remote.

It should be repeated, however, that while it is not difficult to obtain dry and finely pulverized pneumonic sputum under experimental conditions, yet practically the drying of the mass is rarely complete enough to permit thorough powdering, and the particles which are removed mechanically from the sputum, after this fluid has dried on cloth, wood, or metal, are of such dimensions that they cannot be carried for any considerable distance by the air currents ordinarily found in well ventilated rooms, or, if so carried, remain in suspension for a very short time. The contaminated particles are not likely, therefore, to be inhaled even by persons in close contact with the patient, and are still more unlikely to lead to an infection of persons in other rooms or at a distance. Only in structures subject to strong draughts, such as factories or railroad carriages, are air currents likely to be strong enough to render these coarse particles dangerous. The possibilities of infection are reduced to a minimum when the dust particles are blown about in the open air. The dilution is so great and the death of the organisms

¹⁵ *Zeit. f. Hyg.*, 1898, xxvii, 175.

contained in the dust is so rapid that infection cannot be assumed as likely to occur.

Germano¹⁶ studied the effect of drying the pneumococcus with dust by a different technique. The results which he obtained with cultures of the pneumococcus showed that when mixed with sterile dust and dried, the organisms died within two days, unless the drying took place at a temperature below 0° C., when the organisms remained virulent for eight days. Mixed with sand and vegetable soil, the organisms died in two days; mixed with volcanic ash (Tuffboden), and kept moist, the organisms remained alive for six days.

Another group of experiments with another organism, presumably pneumococcus, showed a very considerable variation from the above. The culture mixed with brick dust remained infectious for forty days, either kept moist, dried in the air, or dried over sulphuric acid. Mixed with sand this same organism was infectious for sixty days when kept moist or dried at room temperature, and for fifty days when dried over sulphuric acid. This organism was obtained during an epidemic of pneumonia which occurred in a small village, a number of cases developing about the same time. Germano thinks that possibly the epidemic was due to the long life of this organism in the air.

The results of experiments by the same writer with pneumonic sputum confirmed the facts which have been observed as to the long life of the bacteria when dried in coarse particles. For example, pneumonic sputum mixed with room dust kept moist was virulent for twelve days, kept dry, for twenty days, dried over sulphuric acid, for sixteen days, dried at a low temperature, for eight days. The last results are thus somewhat different from those obtained by Patella and Neisser. Sputum mixed with earth (Humusboden) was virulent for twelve days when kept moist, for one hundred and forty days when dried in the air, and for one hundred days when dried over sulphuric acid. With a low temperature the virulence was retained for only sixteen days.

The writer considers that slight variations in the type of the

¹⁶ *Zeit f. Hyg.*, 1897, xxv, 439; *ibid.*, 1897, xxvi, 66 and 273.

diplococcus may contribute very largely to the length of time during which the organisms can resist drying. It was certainly proven that they remained virulent longer when dried than when kept moist. At low temperatures the short life of the organisms seemed to be conditioned by the fact that the sputum dried slowly at a point near 0° C. so that the bacteria were really kept moist. The rapidity of the drying process at room temperature had no influence upon the life of the diplococcus. Germano concludes, finally, that air infection of human beings by the organism is possible with the pneumococcus, but the chances are relatively small.

It will be seen from the preceding résumé that the views expressed by Cornet on the possibility of dust infection in tuberculosis can hardly be considered as of great import in pneumonia. The sputum of pneumonic patients is often exceedingly viscid and thick; it is not usually produced in the same abundance as in pulmonary tuberculosis; and the coughing of the patients is rarely so prolonged or strongly expulsive. Coarse particles are therefore less likely to be distributed in the neighborhood of such a patient. When such particles are expelled, the results of Sticher and Beninde show that the air currents which are ordinarily present in well ventilated houses are insufficient to remove bacteria from the moist or dried sputum. The risk of infection must be largely confined to those who handle the bedding, etc., of pneumonic patients.¹⁷ The experiments of Neisser and Germano are not wholly consistent, but tend to show that the pneumococcus dies early when dried in finely divided sputum. It is possible that in some of Germano's experiments the organism was not the one which we are accustomed to consider as the pneumococcus.

Conditions under which the Pneumococcus may be Transmitted in Sprayed Particles.—We are largely indebted to Flügge and his co-workers for the experimental investigation of the theory that the transfer of pathogenic bacteria from one person to another is

¹⁷ See in this connection a report by Edson and Ghriskey on "A Hospital Epidemic of Pneumococcus Infections," *Trans. of the Philadelphia Coll. of Phys.*, 1904, xxvi, 6.

possible by the aërial transmission of fine spray particles. They considered that the spraying of fine particles of sputum of saliva by talking, coughing, sneezing, or spitting, might carry infectious material from one person to another if the specific organism remains alive in such spray for a sufficient time to permit the floating particles to be carried from the patient to other persons in the vicinity by the air currents produced for purposes of ventilation.

The studies of this means of transmission of the pneumococcus are not numerous, and we have obtained a large part of our knowledge concerning such transmission by investigating the conditions of infection which obtain in connection with pulmonary tuberculosis. Here again most of the factors are the same with both the pneumococcus and the tubercle bacillus, and we can without impropriety transpose the results obtained by the study of one organism to another.

While it has been repeatedly shown that the air expired from the lungs during quiet breathing contains no bacteria, yet in disease a small number of bacteria may be given off. It has been shown by Koelzer¹⁸ that tuberculous persons by a sort of internal spraying give off tubercle bacilli even during quiet breathing, and that the fine drops containing tubercle bacilli are produced by the pulverizing of the thick mucus in the bronchi during the passage of the expiratory current—the same physical condition which gives rise to the râles heard on auscultation of the chest. Laryngeal tuberculosis increases the liability to the contamination of the expired air. Koelzer obtained positive results in one case out of fifteen persons examined, care being taken to see that no coughing took place while the Petri dishes were exposed to the expiratory current. A similar observation is recorded by Schäffer,¹⁹ who was able to demonstrate lepra bacilli in the expired air of persons suffering from lesions of the nose and throat due to that organism.

The results of many observations on the bacterial content of the expired air have shown, however, that this source of infection

¹⁸ *Zeit. f. Hyg.*, 1903, xlv, 217.

¹⁹ *Arch. f. Derm. u. Syphilis*, 1898, xliii and xlv, 159.

may be practically neglected. On the other hand, coughing and especially sneezing, as previously mentioned, cause an abundant spraying of fine fluid particles which may contain bacteria.

Koeniger²⁰ has studied with great care the conditions under which this spraying takes place. Large numbers of particles are produced when the expiratory stream is interrupted and then suddenly begun. The drops are formed in the portion of the respiratory tract where the stoppage of the air current takes place. Particles produced in the larynx are often stopped by the lips so that the method which the person uses in coughing causes great variations in the number of particles expired in the air. If the mouth be kept nearly closed, the laryngeal particles do not escape in large numbers. If, however, the mouth is tightly closed, drops may be produced from the passage of the air currents over the lips, as the latter are pushed open by the cough. Particles are also sprayed out in clearing the throat. Loud speaking gives more spraying than quiet conversation, and the method of articulation exerts considerable influence. The letters k, p, f, and t cause more spraying than vowels or other consonants. It is probable that more germs are sprayed from a thin, watery sputum than from a thick, mucous variety, but the intensity of the coughing impulse is much more important than the consistence of the sputum. More particles are expelled when the cough is short and sharp. Koeniger's studies on spraying were made by infecting the mouth of the experimenter with *B. prodigiosus* and other saprophytic organisms and then exposing large numbers of plates while talking, coughing, or sneezing.

Dimensions of the Sprayed Particles.—The size of the particles produced by coughing varies greatly. Heymann²¹ studied the size and number of the drops by catching the spray produced by coughing on glass slides and measuring the size of the drops so produced. The finer particles had a diameter when flattened on glass of from thirty to forty micra. Sneezing, according to my own observations, may give rise to a very fine spray, the particles not measuring over ten to twenty micra. The same is true of the

²⁰ *Zeit. f. Hyg.*, 1900, xxxiv, 119.

²¹ *Ibid.*, 1899, xxx, 139.

drops produced in a spray apparatus using either a hand bulb or air at high pressure. Many of the particles are very small and contain no bacteria. Those above twenty micra usually contain one or more organisms if the sputum is rich in bacteria. Even the smaller particles, however, though they may not contain bacteria, when collected and examined are found to have a nucleus formed either by mucus or by salt crystals. The evaporation from these small particles when sprayed into the air is exceedingly rapid, because of their small size.²²

Spatial Distribution of the Sprayed Particles.—Flügge in a series of papers²³ has shown that a person with a cough sprays fine particles into the surrounding air, the radius of the zone of such spraying being usually one meter, rarely two meters. Within this area therefore the air may contain floating particles carrying pathogenic bacteria. Flügge has shown that fine dust particles laden with bacteria may be carried horizontally by a stream of 0.2 mm. per second, one five-hundredth of the speed of a barely perceptible draught. Upward translation of these motes requires a slightly greater wind velocity, about 0.3 to 0.4 mm. per second. Stronger currents of air may carry them to great distances. Hutchinson²⁴ was able to demonstrate the transportation of particles containing *B. prodigiosus* for a distance of 600 meters. The drops produced by coughing, sneezing, etc., are usually larger and heavier than those just mentioned, and Heymann²⁵ has shown that a large proportion of them settle out of the air of an ordinarily ventilated room within an hour. Particles of this size are not transported laterally to any very great extent. In fact, it is exceedingly difficult to demonstrate tubercle bacilli in the air of wards containing tuberculous patients.

As the sprayed particles settle they adhere to the furniture, walls, bedding, and carpets, and dry. It is then impossible to remove them by any stream of air within the limits of ordinary

²² See on this point Thomson, *Conduction of Electricity through Gases*, Cambridge, 1903, p. 135.

²³ *Zeit. f. Hyg.*, 1897, xxv, 179; *ibid.*, 1899, xxx, 107; *ibid.*, 1901, xxxviii, 1.

²⁴ *Ibid.*, 1901, xxxvi, 223.

²⁵ *Ibid.*, xxxviii, 21.

ventilation currents. It is possible to remove these particles, however, by dry brushing, sweeping, or dusting, and the powder so formed may float for a long time in the room or be transported to adjacent ones.

Bacterial Content of Sprayed Particles.—Direct evidence of the bacterial content of the sprayed sputum has been obtained by B. Fränkel, who examined the contents of two hundred and nineteen face masks each of which had been worn for twenty-four hours by persons whose sputum had contained tubercle bacilli. In twenty-six of these masks tubercle bacilli could be demonstrated. Fränkel assumes that in thirty-two days 2600 tubercle bacilli had been caught in masks which would otherwise have escaped into the air. In a considerable number of cases of tuberculosis, however, Fränkel pointed out that the masks remained uninfected so that only a small number of patients could be shown to cough out drops of sputum or saliva containing tubercle bacilli.

The presence of virulent organisms in sprayed sputum has been verified by other observers.²⁶ The most detailed study is perhaps that of Heymann,²⁷ who examined with especial care the conditions attending the spraying of tuberculous sputum by patients under, so to speak, natural conditions—that is, the coughing was not forced, the patients simply being confined to a room while the tests were carried out. The particles sprayed out by the patients were collected and found to contain abundant tubercle bacilli.

An interesting example of the large numbers of bacteria which may be expelled is that reported by Schäffer²⁸ where one leprosy patient at a single sneeze gave off 25,000 bacilli, and another patient, 110,000. Patients with severe lesions of the tuberculous type gave off from 10,000 to 185,000 leprosy bacilli in ten minutes' talking. The sprayed bacteria were caught on slides placed close to the patients' mouths and only a very few could be demonstrated at a distance of one and one-half meters.

²⁶ v. Hübener, *Zeit. f. Hyg.*, 1898, xxviii, 348; v. Weismayer, *Wiener klin. Woch.*, 1896, p. 1039; Bing, *Zent. f. innere Medizin*, 1905, p. 54; Mendes de Leon, *Arch. f. klin. Chir.*, 1904, lxxii.

²⁷ *Zeit. f. Hyg.*, 1901, xxxviii, 21.

²⁸ *Arch. f. Derm. u. Syphilis*, 1898, xliii and xliv, 159.

Hamilton²⁹ finds that streptococci are expelled from the mouth by coughing or even by breathing by persons with streptococcus infection of the upper air passages.

Life of the Bacteria in the Spray Particles.—In order to study the length of life of bacteria in sprays, Laschtschenko³⁰ atomized diluted pneumonic sputum (ten parts sputum and from one to two parts water) in a closed vessel and caused the particles to be carried upwards for one meter by a current of air of from 6 to 10 mm. per second.

The spraying was continued under low pressure for one and one-half hours. The particles were collected and the fluid injected into mice, with three positive and two negative results. Spraying *undiluted* sputum with air speeds of 10 to 12 mm., he obtained one positive and six negative results, the infectious nature of the sputum being previously determined by injecting mice. Using the same apparatus and conditions with phthisical sputum, a positive result was obtained in all cases with air speeds of from 6 to 14 mm. per second, the sputum being diluted and undiluted. The spray was produced by a very low air-pressure stream. The results show that the pneumococcus and the tubercle bacillus can live for a longer or shorter time in sprayed sputum. The writer gives no explanation of the fact that many more positive results were obtained with tuberculous sputum than with the pneumococcus, but it is evident from my own experiments, to be given later, that the drying which the pneumococci underwent while carried up in the air current was sufficient to kill many of the organisms.

A phenomenon noted by Koeniger³¹ is of interest in this connection. He observed that after spraying large quantities of cultures of *B. prodigiosus* over the floor and furniture of a room it was impossible to obtain colonies of this organism on exposed plates even when large amounts of dust were produced by energetic brushing. Growths were obtained from many other organisms but not from the *prodigiosus*. This fact was not fully understood by Koeniger, who states that the results of his

²⁹ *Jour. of the American Med. Assoc.*, 1905, p. 1108.

³⁰ *Zeit. f. Hyg.*, 1899, xxx, 133.

³¹ *Ibid.*, 1900, xxxiv, 119

experiments show that the bacteria must be moist to produce a growth. The true explanation was given shortly afterward by Kirstein, who showed that the reason for the negative results was that *B. prodigiosus* when sprayed in fine particles dried rapidly and was promptly killed, especially when exposed to diffuse daylight. In one set of experiments by the latter, *B. prodigiosus* was sprayed in two rooms and the falling germs caught on glass plates. In the dark room the bacteria remained alive for fifteen days, in the well-lighted room for only three days.

Similar results were obtained by Kirstein³² for pathogenic organisms such as the typhoid bacillus, which remained alive for only a few hours, the tubercle bacillus, which was alive for from four to eight days in diffuse light and as long as forty days in the dark.

Staphylococcus pyogenes aureus and *streptococcus* remained alive for from ten to sixteen days; diphtheria bacilli, less than twenty-one hours; anthrax bacilli, nearly ten weeks. The pneumococcus was not investigated, as the author assumed from the results of previous studies by Neisser and others that prompt death of the pneumococcus occurred after drying.

In a more recent paper, Kirstein³³ finds that the tubercle bacillus lives for from eight to fourteen days when sputum is sprayed on fine dust, from four to seven days when tuberculous sputum is finely powdered, five days when deposited on fine cloth fibers, and finally that the bacillus lives but three days on fine street dust although it was alive for eight days on coarse dust of the same variety. In all of these tests the bacteria were exposed to diffuse daylight.

All observers are agreed then that the life of the bacteria when sprayed and dried may be safely assumed to be much shorter than when they are dried in masses. Diffuse light and especially sunlight rapidly destroy the organisms, while preservation in a dark, cool place tends to prolong their existence.

³² *Zeit. f. Hyg.*, 1900, xxxv, 123; *ibid.*, 1902, xxxix. See also Ficker, *Zeit. f. Hyg.*, 1898, xxix, 1.

³³ *Zeit. f. Hyg.*, 1905, 1, 186.

Summary.—As will be seen from the survey of the bibliography of the subject just given, the conditions of the viability of the pneumococcus have been fairly well established when either sputum or other fluids containing the organism are dried in bulk and exposed to diffuse daylight or the direct rays of the sun. There are minor inconsistencies in the results dependent upon the method used, the sensitiveness of the animal employed to determine the presence of living pneumococci, and possibly also upon slight variations in resistance of the various strains. The identification of the organism was, however, so far as is reported in many of the studies, entirely dependent upon either the morphology of the bacteria isolated or even upon the death of the animal without any microscopical verification of the presence of a septicæmia. As it has been shown that there are other capsulated organisms which are fatal to mice if given in sufficiently large amounts, and as these animals and also rabbits frequently die after the injection of sputum without the presence of pneumococci being determinable either morphologically or by culture, it seemed to the writer that a few experiments might properly be devoted to a repetition of the studies of the earlier Italian and German workers whose papers have already been considered. The experiments of Germano in mixing cultures of the pneumococcus or sputum with sterile dust were not repeated for they are very complete as they stand and are not especially pertinent to the question in hand.

The main portion of the writer's studies were therefore devoted to the investigation of the question of fine sprayed particles containing pneumococci and the length of life of the organisms in this spray. This ground has not been fully covered by previous workers, and as its great importance in the transmissibility of the tubercle bacillus has been shown, it seemed proper to extend our knowledge to the pneumococcus although it has generally been assumed that that organism was too sensitive to desiccation to live very long in fine particles.

I.—EXPERIMENTS ON THE VIABILITY OF THE PNEUMOCOCCUS IN
LARGE MASSES OF SPUTUM.

EXPERIMENT I.—The following tests were made to determine the viability of the pneumococcus in sputum when kept moist and at room temperatures and also when kept at 0° C.

TABLE I.
TESTS WITH MOIST SPUTUM.

Day of Test.		1	5	10	15	20	30	42	60
I. Thick, mucous sputum from 3d day of pneumonia	22° C.	+	+	o	o	o	o	o	o
	0° C.	+	+	+	+	+	+	+	o
II. Thick, rusty sputum from 3d day of disease....	22° C.	+	+	+	o	o	o	o	o
	0° C.	+	+	+	+	+	+	o	o
III. Thin, fluid sputum from 5th day of disease.....	20° C.	+	+	+	+	+	o	o	o
	0° C.	+	+	+	+	+	o	o	o
IV. Thin, yellowish sputum after crisis.....	20° C.	+	+	o	o	o	o	o	o
	0° C.	+	+	+	+	+	+	+	o
V. Thick, yellowish sputum from 8th day of disease...	20° C.	+	+	+	+	o	o	o	o
	0° C.	+	+	+	+	+	+	o	o

The positive marks mean that the pneumococcus was either isolated from the sputum by culture, or, especially after the 5th day, that the subcutaneous injection of from one fourth to one fifth of a cubic centimeter of the undiluted sputum was fatal to a mouse. No result was considered as positive unless capsulated, Gram-positive organisms could be isolated from the blood of the animal, and unless the coccus fermented inulin after plating out on chest-serum agar. Occasionally by the use of very large quantities of sputum (0.5 to 1.5 c.c.) it was possible to kill mice up to fifty days, but often only one animal out of three died, showing that only a few organisms remained alive.

It will be seen from the table that the life of the pneumococcus in fresh, moist sputum at room temperatures is rarely over two weeks. The specimens were kept in the dark in order to compare them directly with those at 0° C., which were of necessity inclosed in a cold-storage box. Two specimens kept in strong diffuse daylight lost virulence for mice in less than five days.

The rapid death of the organisms in sputum as compared to chest-serum is possibly due to the bactericidal action of the mucus of the sputum.

EXPERIMENT II.—Tests were also made by drying sputum in Petri dishes at room temperatures. Some of the specimens were kept in a dark, dry spot, others were exposed to diffuse daylight in a room facing the south, others were exposed to full sunlight. Fragments of the dry crust of sputum were then removed, rubbed up in sterile bouillon, and inoculated into mice. Other specimens

It is evident from the table that the exposure of the pneumococcus to sunlight results in the prompt death of the organism. The mere powdering of the sputum also destroys the pneumococcus, a phenomenon probably due to the rapid and complete drying which takes place. The action of even diffuse daylight in hastening the death of the organism is evident from the table. Exposure of the powder to sunlight effects an even more rapid destruction, there being probably three factors in the process. One is the formation of oxidizing agents, probably hydrogen peroxide, by the action of the sun's rays upon the traces of moisture remaining in the sputum,³⁴ a second the rapid drying which takes place, and a third, the destructive action of the chemical portion of the sun's rays.

EXPERIMENT III.—Sputum was spread on fragments of sterile wood, and tin, and on woollen and cotton cloth. The specimens were allowed to dry, and were kept either in diffuse daylight or sunlight. The life of the organism was about the same on wood and tin as on glass. On cloth several tests gave a slightly longer life, the sputum being virulent for mice after sixty days. This is explained by the penetration of the cloth which takes place when soaked with sputum, the fiber of the cloth protecting the organism from light and the layer of sputum formed being thicker than on a flat surface. This effect was more marked in those fragments exposed to sunlight, one piece of woollen cloth being virulent to mice after twelve hours' exposure, about six hours being given on two successive days in May. The death of the bacteria occurred on two hours' further exposure.

II.—VIABILITY OF THE PNEUMOCOCCUS IN FINE SPRAYED PARTICLES.

Technique.—In order to spray sputum and other fluids containing pathogenic bacteria and to collect the finer particles, it is necessary to conduct the operation in an air-tight chamber, to avoid contamination of the laboratory and infection of the operator.

The apparatus employed by the writer was modelled upon the one described by Kirstein,³⁵ with some slight modifications. The box was constructed of seven-eighths inch white wood lumber with internal measurements of 38 cm. in depth, 35 cm. in width, and 152 cm. in length. At one end were perforations

³⁴ Bie, *Mitth. aus Finsens Med. Lysinstitut*, 1905, Neuntes Heft, p. 5.

³⁵ *Zeit. f. Hyg.*, 1900, xxxv, 145.

for the insertion of the tip of the spraying apparatus and apertures to permit of the escape of air driven into the chamber while spraying the sputum. In order to prevent direct carrying of particles the full length of the chamber and the deposition of the organisms in coarse masses upon the Petri dishes or other substances used to collect the spray, two baffle plates were placed about the middle of the chamber, 22 cm. apart. These plates were of glass and measured 28 by 35 cm. They were held in place by narrow strips of wood nailed on the inner side of the box, and further secured by putty and a layer of enamel paint. The plate nearer the spraying apparatus was so placed that its upper portion was in contact with the lid of the box. The plate farther from the spraying apparatus was in contact with the floor of the box, leaving a space of 10 cm. between its upper edge and the lid (see Fig. 1). It was thus impossible for particles from the spray to pass directly from one end of the box to the other. The coarser masses strike the first plate and adhere to it. Only the finely suspended particles pass over the top of the second plate, and this only when a current of air is drawn through the apparatus (see Experiment IV).

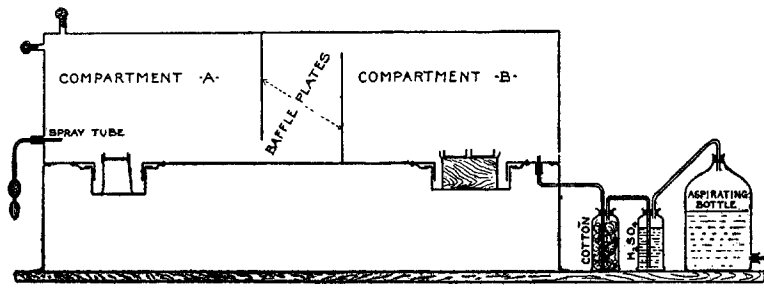


FIG 1 Diagram of Box for Spraying Pathogenic Bacteria.

In order to collect the sprayed particles, three apertures were made at the bottom of the box, two of which were circular, measuring 13 cm. in diameter; the third was rectangular and measured 26 by 14 cm. These openings had a tin collar inserted in them extending about 10 cm. below the bottom of the box. These collars were rendered air-tight by white lead. Each opening was closed during the experiment by placing under it a dish some 3 or 4 cm. larger in diameter than the collar, the dish being filled with 1:1000 mercuric chloride solution, thus making a water seal. The dishes were held in position by the use of small cupboard buttons which could be swung into place under the rim of the dish. Before the spraying was commenced, suitable receptacles for catching the spray, such as Petri dishes, either dry or containing culture media, or fragments of sterilized cloth, wood, tin, etc., were placed on small stands which rested on the bottom of the dish. For the smaller apertures in the first compartment these stands were ordinary drinking-glasses which were inverted and the upper end surrounded by a strip of half-inch surgical adhesive plaster. The Petri dish placed on this adhered quite firmly, and there was no danger of its falling off during the process of removal, even though the glass was consider-

ably tipped. In the larger rectangular opening the stand was made of half-inch pine board with four nails for legs, surrounded by a collar of adhesive plaster. This collar retained the plates in position and prevented their shifting during insertion or removal of the stand. The surface of the Petri plate when inserted was approximately level with the bottom of the spraying-box.

In order to render the inside of the box air-tight and waterproof, the corners were filled with putty and the inside was given three coats of thick enamel bath-tub paint. The lid was held in position by one-eighth inch steel wires which passed from a turn buckle fastened to the lid on one side, underneath the box and over a wooden brace to a turn buckle on the opposite side. The turn buckles could be screwed tight, thus holding the lid firmly in position. In order to make a suitable seal, the upper edge of the box was smeared with a thick layer of paste sold commercially as anti-phlogistine. This was found to be better than putty or white lead, as it did not set, but remained moist and somewhat pliable for a period of nearly two months. At the end of the box farthest from the spraying apparatus was an aperture similar to those in the spraying end of the box. A suitable opening was made by boring a hole from the outside of the box, about 22 mm. in diameter. A collar, about 3 mm. in width, made of wood, was left on the inside of this opening. Short pieces of brass tubing were heated over a Bunsen flame, smeared with rosin, and quickly inserted in the holes, into which they fitted snugly, the collar of wood which was left insuring a firm seat. As soon as the rosin cooled, an air-tight joint was obtained. Four of these apertures were made: one for the insertion of the spray tube, one for aspiration of the air current, two for egress of the air forced in by the pressure apparatus. The spray was produced by a long glass spray tube which was inserted through one of the openings and tightly packed in position with absorbent cotton. After the spraying was completed, these tubes could be easily sterilized by boiling in one per cent. sodium carbonate solution. The spraying was done by means of compressed air, the pressures used varying from five pounds or less to the square inch to forty pounds to the square inch. It was found necessary to use a higher pressure in the case of thick, mucous sputum than for thin, watery sputum. By this means suitable quantities of thick sputum, in many instances 30 to 40 c.c., could be atomized during the course of ten minutes. Before beginning the experiments the box was tested by closing all the apertures with corks and inserting a water manometer in one of the openings and forcing in air through another. A pressure of three inches of water was sustained for fifteen minutes, showing that the box was air-tight for this pressure. Higher than this it was impossible to go because the water-seals would have been forced by the pressure. As it was, some difficulty was experienced in spraying into the apparatus unless air was being drawn out at the same time, because of the escape of bubbles of air through the water-seals. It would be advisable, therefore, to modify the apparatus and make the seals somewhat deeper; possibly 15 cm. would be better than the 10 cm. used. Air was drawn through the box by means of an aspirating bottle graduated in liters. In order to catch any bacteria and prevent their entry into the aspirator, a bottle was inserted between the box and the aspirator containing tightly packed absorbent cotton, and a second containing sulphuric acid through which the aspirated air was forced to bubble. The rate of aspiration could be measured

by timing the rate of outflow from the bottle. The speed at which the particles were carried from the chamber nearer the spraying apparatus, which may be for convenience termed A, into the chamber farther from the spraying, called B, could be determined as follows. (Fig. 2.)

The distance between the two baffle plates being 22 cm., their height 28 cm., the length of the hypotenuse would be about 36 cm. The diameter of the channel is then approximately 6 cm. The cubic contents of this channel from the lower aperture of the first baffle plate to the upper aperture of the second would be 36 by 35 by 6 cm. This is approximately 7560 c.cm. If this amount of air is aspirated from the farther end of the box in one minute, the velocity in the channel will be 6 mm. per second, which is near the lowest limit of air speed which will move very fine particles. Air speeds, therefore, were used in these experiments of from 2 to 10 mm. per second.

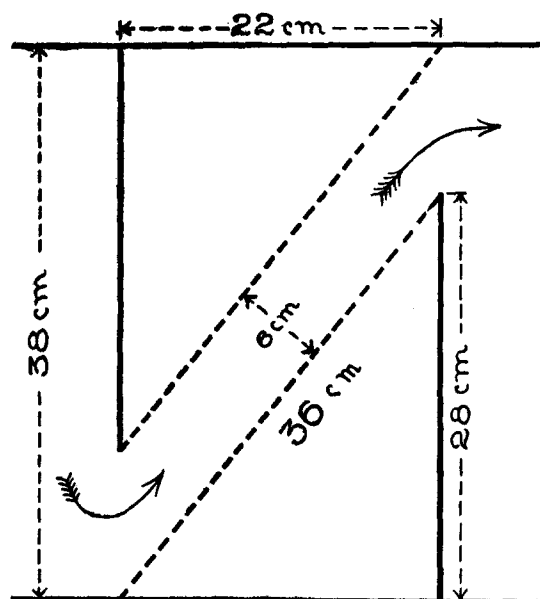


FIG. 2. Diagram of Dimensions and Course of Air Current in Spraying Box.

EXPERIMENT IV.—A preliminary experiment made with bouillon cultures of *B. prodigiosus* showed the box to be tight and that no bacteria passed the baffle plates unless a current of air was drawn through the box.

Sputum was then obtained from a case of acute lobar pneumonia at about the third day of the disease. Morphological examination showed numerous diplococci in nearly pure culture. They were positive to Gram, but no capsules were demonstrable. The sputum was plated on chest-serum agar and diplococci isolated which were Gram positive, had well marked capsules, fermented inulin, and killed a mouse in three days, capsulated cocci being found in the heart's blood.

Ten cubic centimeters of this thick sputum were sprayed in the box, using 40 pounds air pressure. During the spraying and for some time after, a slow current of air was drawn through the apparatus at a rate of about 0.2 mm. per second. Cover-slips exposed in the second compartment during spraying showed numerous particles derived from the spray, some of which contained pneumococci or the other bacteria of the sputum. A current of 0.2 mm. per second is therefore capable of transporting spray-carrying bacteria for a distance of at least one meter.

After the spray had been stopped, covers were exposed every fifteen minutes for two hours. At the end of an hour most of the particles carrying bacteria had settled. Covers exposed after ninety minutes had elapsed showed no bacteria, only very small particles of mucus taking a blue stain with gentian violet. This shows that the bacteria may be assumed to settle 38 cm. in from sixty to ninety minutes.

In order to study the settling of the particles more conveniently and to determine the length of time for which sprayed sputum particles can remain in suspension, ten cubic centimeters of this very thick sputum were sprayed at 40 pounds pressure into a tall aspirating jar about 45 cm. in height, the air contents of which had been cleansed of dust by aspiration through a thick cotton plug. A jar was used for this preliminary experiment instead of the box just described, because of the ease with which suspended particles could be rendered visible by a strong beam of light. A thick fog of the sprayed particles was produced which remained suspended for sixteen hours and could be rendered easily visible by passing a beam of light from an electric arc. At the end of twenty-four hours only a few fine particles could be seen on concentrating the light with a lens. No bacteria were deposited on cover-glasses or culture plates after the jar had stood for two hours.

In order to determine whether the fine spray particles, which remained a long time in suspension after spraying a broth culture, contained bacteria, the jar was filled with spray from a bouillon culture of *B. prodigiosus*, and after standing one hour the plug was removed from the upper end and the bottle was reversed and allowed to rest on the mouth of a large battery jar. Under the mouth was placed an agar covered Petri dish. At the end of one hour the dish was removed, covered, and allowed to remain at room temperature for several days. Abundant growth took place.

In a repetition of this experiment growth was obtained by allowing the fog to settle on plates exposed at the end of one hour and thirty minutes, but no growth was obtained after two hours, nor after four and six hours. This agrees with the results obtained by Stern,³⁶ who states that ordinary dust particles settle in still air in from one hour and a half to three hours, and can only be kept afloat by air currents of from ten to thirty millimeters per second. Very fine particles still containing bacteria can

³⁶ *Zeit. f. Hyg.*, 1889, vii, 44.

be transported laterally by a current of 0.2 mm. per second, and kept afloat by a current of from 0.3 to 0.4 mm. per second.³⁷ The fog made evident by the light beam after a period of from five to six hours is probably composed of dried salts and albumin or mucus particles, and does not contain bacteria.

Air currents which cause the movements of these very fine particles have been shown to be of much less velocity than those which occur in well ventilated rooms where the motion is from 1 to 2 mm. per second. Air at ordinary temperatures does not produce a perceptible draught until its velocity reaches 10 cm. per second. In unventilated rooms the current is less than 0.6 mm.

It is therefore possible for spray particles containing the pneumococcus to float in the air of an unventilated room for some three hours, if we assume the rate of fall as determined by the experiments to be at least 30 cm. per hour and the head of the patient to be about one meter from the floor. With air currents of very slight intensity, however, the finer particles may be carried for considerable distances. Many of these fine droplets do not contain bacteria, so that the practical danger from a patient with pneumonia is less than appears from tests under artificial conditions. The coarse particles containing many bacteria fall rapidly, and in the case of the pneumococcus, as will be seen later, many of the suspended organisms lose their vitality in the course of one or at most two hours.

EXPERIMENT V.—In order to avoid the use of mixed cultures such as would be obtained from sputum, a pleuritic fluid containing enormous numbers of pneumococci was also employed in the studies. This fluid was obtained by injecting small amounts of sputum into the right pleural cavity of large rabbits. The injection is easily made by passing a fine needle through one of the intercostal spaces on the lateral aspect of the thorax. The animals usually die in two to three days, and if the thorax is carefully opened from 10 to 50 c.c. of clear or slightly bloody fluid can be obtained. Usually both pleuræ and the pericardial sac contained fluid.³⁸

Ten cubic centimeters of the pleuritic fluid were sprayed, and at the same time sixteen liters of air were removed at such a rate that the velocity between the baffle plates was 2 mm. per second. The control plate in Chamber A was

³⁷ Flügge, *Zeit. f. Hyg.*, 1897, xxv, 193.

³⁸ This method of obtaining a fluid rich in pneumococci was suggested to me by Dr. A. B. Wadsworth. The organisms remain alive in the serum for a long time at 0° C.

removed after thirty minutes and was found to be slightly moist. Bouillon was poured on the surface and rubbed up with a platinum needle. Loops from the bouillon were then transferred to chest-serum agar. An abundant growth of pneumococci was obtained. Plates exposed in Chamber B were removed at the end of an hour and a fresh set inserted. Cover slips which had been exposed during the same time showed numerous capsulated cocci. Bouillon was poured over one plate, rubbed up with a platinum spatula, and injected into a mouse. The animal died from pneumococcus infection. Other plates from Chamber B were exposed one, two, and three and a half hours to diffuse daylight. At the end of this time mice were inoculated from the two-hour plates, and two rabbits³⁹ were injected in the ear vein with an emulsion from two three-and-a-half-hour plates. One mouse died twenty days later, but no pneumococci could be recovered. The other lived for two months. The rabbits did not die. A third plate after three and a half hours was covered with chest-serum agar, but no growth was obtained. Another plate from Compartment B was exposed three hours to sunlight on a slightly overcast day. Plates were made after emulsifying with bouillon and three mice injected. There was no growth on plates. One mouse died twelve days later, but no pneumococci could be demonstrated. The others did not die. Another plate was dried over calcium chloride for three hours in diffuse light. Plate cultures and animal inoculations were negative.

The results of this experiment may be considered as showing that spraying a thick albuminous fluid containing pneumococci and allowing the fine spray to dry on glass is fatal to the organisms in a very short time. If drying is prevented by collecting the particles as they fall on moist chest-serum agar, growth will be obtained if the bacteria have not been in suspension over ninety minutes. A fluid of the type used corresponds pretty closely to the thin, serous sputum of certain cases of pneumonia.

EXPERIMENT VI.—Twenty-five cubic centimeters of the thick sputum used in Experiment IV were sprayed in the box during ten minutes, using an air current of about 6 mm. per second. Control plates from Compartment A were positive. Plates from Compartment B removed immediately were negative to mice, and cultures gave only staphylococci. Cover-glasses showed numerous drops varying from three to fifteen micra in diameter. The larger drops frequently contained two or three diplococci.

One hour after spraying, cover-glasses were placed in Compartment B and allowed to remain fifteen hours. These showed only a few bacteria, less than one per square centimeter. There were, however, many masses of mucus which had fallen on the slide, most of which did not contain bacteria. No free organisms were found, all were surrounded by more or less mucus.

³⁹ It was thought, inasmuch as the pneumococci used had been adapted to rabbits, that these animals might be more susceptible to infection than mice.

Chest-serum-agar plates, exposed in Compartment B for a period beginning one hour after the spraying was finished, gave abundant growth of staphylococci, but mice injected with an emulsion showed no pneumococci. Some plates remained in Compartment B for a number of days, but no pneumococci could be demonstrated. Dry plates remained ten days, and when covered with agar gave numerous colonies of *Staphylococcus pyogenes aureus*. Mice which were injected died, but no pneumococci could be isolated. Plates from this spraying were kept in the dark and their contents injected into mice at various intervals. Some of the animals died, but no pneumococci could be obtained.

A plate dried over calcium chloride for fifteen hours in the dark gave no pneumococci, but only staphylococci.

The results of the experiment show the rapidity with which the pneumococcus dies when sprayed in fine particles and allowed to dry. The drying seems to be an important factor, for if, as is shown in Experiment V, the bacteria are caught on moist media, a growth will be obtained. As a rule also we must assume that there are other factors at work, for the viability of the organisms after spraying sputum is certainly less than that after spraying rabbit chest-serum. The action of the mucus must be considered and possibly also the osmotic relations of the organism to the sputum may affect the pneumococcus unfavorably. It is also not impossible that a considerable proportion of the pneumococci in sputum from the later stages of the disease are not viable.

EXPERIMENT VII.—Forty cubic centimeters of fresh, thin, serous sputum, from the sixth day of the disease, one loop of which was capable of killing a mouse in forty-eight hours, were sprayed for one hour with an air current of 10 mm. per second. The control plate from Chamber A killed a mouse in forty-eight hours. Pneumococci were isolated which were positive to Gram, were capsulated, and fermented inulin. The plate was dry when it was removed from the compartment.

Plates from Chamber B, removed at the end of spraying and found to be dry, were washed with bouillon and the washings injected into two mice. One died, but no pneumococci were found; the other lived a month. Washings from these plates were sown on the surface of chest-serum agar in order to avoid the inhibiting action of the anaërobic conditions which exist under a layer of agar. Staphylococci and other unidentified organisms were obtained, but no pneumococci.

Plates dried over calcium chloride were also negative as regards pneumococci. These experiments were repeated with sputa from different cases and at different times of the disease, but the results were practically the same.

It is evident that finely sprayed sputum contains no viable pneumococci after drying on glass for one hour. The positive results occasionally obtained from the Control plates in compartment A may be explained by the thick layer of sputum which is deposited and prevents complete desiccation. The results obtained by using thin, serous sputum do not vary from those obtained when thick, mucous sputum was sprayed, although differences appear when the sputa are dried in bulk.

EXPERIMENT VIII.—The technique was varied slightly so as to transfer large numbers of the organisms and thus to keep them moist. About 100 c.c. of pleuritic fluid were sprayed, with an air current of 10 mm. per second. Fifteen minutes after spraying was completed the plates in Compartment B were removed. They were still moist. A mouse injected with an emulsion of the deposit died with pneumococci in the heart's blood. A plate was exposed to sunshine for twenty minutes, during which time it dried. A mouse injected did not die. A plate of the same series was dried over calcium chloride for thirty minutes. One-half was injected into a mouse which died with pneumococcus sepsis. The plate was dried thirty minutes more; mouse died with pneumococci in the heart's blood. A plate from the same series was dried two hours over calcium chloride; a mouse injected did not die. A broth culture was made from this plate and showed pneumococci which killed a mouse (see results obtained by Ottolenghi). Another plate was dried for three hours and a mouse injected and a broth culture made. Broth was negative and the mouse remained alive. Plates dried in air for one hour were positive; for one and a half and two hours, negative.

It is possible that when only a small quantity of fluid is sprayed there are not enough virulent pneumococci left after drying to kill the experimental animal. A certain minimum dose seems necessary to kill even as susceptible an animal as a white mouse. A larger quantity of fluid was therefore sprayed in this test, and as shown by the slightly longer life of the pneumococci as compared to Experiments V, VI, and VII, the quantity exerts some influence. The conditions approach those which occur in drying sputum in bulk (see Table II) where the life of the organism is considerably prolonged.

During this test fragments of sterilized woollen and cotton cloth, tin, and wood were exposed in Compartment B. They were removed, allowed to dry in the air for thirty minutes, and scrapings from the surface tested. The organisms on the tin and wood were dead, those on the cloth were alive, but died on drying for thirty minutes longer.

EXPERIMENT IX.—A number of observers have thought that the pneumococcus in sputum is rapidly destroyed by the bactericidal action of the mucus of the sputum. Such action has been shown to take place with nasal and uterine mucus and pure mucin.⁴⁰

⁴⁰ Wurtz and Lemoyez, *Compt. rend. de la Soc. de Biol.*, 1894; Arloing, *Jour. de phys. et de path. gén.*, 1902, iv, 291 (Bibliography).

(a) This was tested by keeping a thick, mucous sputum at 0° C., as recorded in Experiment I. As the pneumococcus dies on culture media or in rabbit serum in a few days unless kept at 0° C., it was thought that a better differentiation could be obtained by working at the lower temperature and in the dark. When first collected the specimens killed mice in doses of a few cubic millimeters in forty-eight hours. After fifteen days at 0° C., a much larger amount of sputum was required to kill a mouse of about the same size as that used during the first experiment. At the end of six weeks mice often could be killed only by doses of a cubic centimeter of pure sputum, while one specimen was no longer virulent after twenty days. Evidently a large number of the pneumococci die in two weeks when kept in moist sputum.

As it is well established that the pneumococcus remains alive for a long period when kept in serum mixtures at 0° C., a combination of this fluid with sputum should retain its virulence as long as pure sputum unless some bactericidal agent is present in the sputum. Such a mixture was therefore made and kept in Petri dishes. The sputa used were the same as in Table I. The results were as follows:

TABLE IV.
TESTS WITH SPUTUM-CHEST-SERUM MIXTURES KEPT AT 0° C.

Day of Test.	1	5	10	15	20	30	42	60
Sputum No. I.: + serum containing pneumococci.	+	+	+	+	+	+	+	0
Sputum No. II.: + serum containing pneumococci.	+	+	+	+	+	+	0	0
Sputum No. III.: + serum containing pneumococci.	+	+	+	+	+	+	+	0
Sputum No. IV.: + serum containing pneumococci.	+	+	+	+	+	+	+	0
Sputum No. V.: + serum containing pneumococci.	+	+	+	+	+	0	0	0
Serum alone.	+	+	+	+	+	+	+	+

The table shows that the serum-sputum mixtures do not retain their virulence for mice much longer than the original unmixed sputum as given in Table I. In two cases, however, that of sputum No. III and No. IV, virulent pneumococci were still present at the end of six weeks, while the pure sputum was non-virulent after three weeks' preservation. This difference is possibly due to the fact that but little mucus was present in the sputum. The practical importance of these findings is that the

thin, serous sputa are likely to retain their infectious qualities somewhat longer than the thick, mucous specimens, and as the thin sputa are most easily sprayed during coughing, special care should be taken to avoid contact infections.

In order to determine the action of the mucus during spraying and after drying of the spray particles, the following experiment was planned.

(b) Specimens of sputa Nos. VI and VII were mixed with an equal quantity of rabbit chest-serum rich in pneumococci. Mice injected with the mixture died promptly of pneumococcus infection. The specimens were kept on ice in the dark, and in diffuse daylight at room temperature. The results were as follows:

TABLE V.
TESTS WITH SPRAYED SPUTUM-CHEST-SERUM MIXTURE.

		Sprayed after				
		2 days.	4 days.	8 days.	15 days.	20 days.
Sputum VI.: + serum on ice in dark. Thin, serous sputum.	Plates removed from Comp. B. immediately.....	+	+	+	+	+
	Dried in air for 30 min..... " " sunlight for 30 min.	+	o	o	-	-
Sputum VI.: + serum in dark at room temp.	Same conditions as above.	+	o	-	-	-
		o	o	-	-	-
Sputum VII.: + serum on ice. Thick mucous sputum.	Same conditions as above.	+	+	o	-	-
		o	o	o	-	-
Sputum VII.: + serum at room temp. (18°-22° C.).	Same conditions as above.	o	o	o	-	-
		o	o	o	-	-
Sputum VI.: Without admixture on ice.	Same conditions as above.	+	+	+	o	-
		o	o	o	-	-
Sputum VII.: Without admixture in dark at room temp.	Same conditions as above.	+	o	o		
		o	o	o		
Chest-serum on ice....	+	+	+	+	+
Chest-serum in light...	+	+	+	o	-

The results of the experiments show that pneumococci die off in mucous sputum more rapidly than they do in a serum mixture and that this action is probably due to the mucus present. The pure serum used in this test preserved its virulence for weeks when kept on ice, and for eight days in diffuse light.

EXPERIMENT X.—In order to determine whether the rapid death of the sprayed organisms is due to the drying which takes place while they are suspended in the air or after they are deposited on the glass plates or other dry substances used to collect them, the following variation was made in the test.

Thirty cubic centimeters of pleuritic fluid were sprayed, using an air current of 10 mm. per second. The contents of the control plates removed at the end of the spraying killed mice in two days and gave an abundant growth on serum-agar. Plates of serum-agar were inserted in Compartment B at the completion of the spraying and the air current was continued for thirty minutes. These plates were removed in thirty minutes and a second set of serum-agar plates was substituted. These were also removed in thirty minutes and a fresh set substituted.

On the first group of plates there was an abundant growth of pneumococci which killed mice—i. e., had lost none of their virulence. The second set of plates showed about twenty colonies each. These were, of course, derived from bacteria which had been in suspension for at least thirty minutes. The third set inserted at the end of an hour after the spraying had ceased and allowed to remain for three and a half hours, showed one or two colonies of pneumococci. Cover-glasses inserted at the same time showed no demonstrable pneumococci after a long search and only small masses of deposited spray. It is evident that practically all the bacteria had settled out from a height of 38 cm. in an hour's time, and that those in suspension for that time were still alive, probably owing to their being protected from complete desiccation by the inspissated serum surrounding them. In order to extend the time during which the organisms could be suspended in the air, a sputum-chest-serum mixture was sprayed into a tall aspirating jar some 45 cm. in height. As the rate of fall in still air of fine particles containing pneumococci is about 40 cm. per hour, the jar was inverted every fifteen minutes for two hours, during which time it was exposed to diffuse light. It was then fixed mouth downward over a Petri dish containing chest-serum agar and was left for six hours in the dark. Numerous colonies of *Staphylococcus pyogenes aureus* developed, but none of the pneumococcus.

As shown above, the organism is alive after an hour's suspension. A second test showed that only a few pneumococci survive for ninety minutes when suspended in a fine spray in diffuse light. Such a fact is of the greatest importance from a point of view of the hygiene of those in close contact with persons suffering with pneumonic infections. It demonstrates the necessity of an abundant air supply to dilute the cloud of organisms which sur-

round a patient with a severe cough. A repetition of the same test allowing the jar to stand in direct sunlight for fifteen and thirty minutes, and then removing it to a dark room to permit the organisms to settle, showed in a very striking manner the value of sunlight as a disinfectant. Only a few colonies of the pneumococcus were obtained after fifteen minutes, and none at the end of half an hour.

SUMMARY AND CONCLUSIONS.

I. In moist sputum kept in the dark at room temperatures the average life of the pneumococcus is eleven days, though considerable variations may be noted in different specimens of sputum.

In the same sputum kept at 0° C. the average life of the organism is thirty-five days.

In sputum kept at room temperature and in a strong light the pneumococcus lives less than five days.

II. In dried sputum (*a*) in the dark the pneumococcus lives on an average thirty-five days; (*b*) in diffuse light, thirty days; (*c*) in sunlight, less than four hours.

III. In powdered sputum even when kept in the dark the death of the pneumococcus takes place in from one to four hours. When exposed to sunlight death occurs within an hour.

IV. No important differences were noted in the life of the pneumococcus when dried on glass, tin, or wood. On cloth the life was usually slightly longer than on non-absorbing surfaces.

V. Sprayed sputum particles remain in suspension for twenty-four hours, but all masses of a size sufficient to contain bacteria settle at a rate of about 40 cm. per hour.

VI. When sputum containing pneumococci is sprayed the organisms rarely survive for more than an hour, and often die in less time. The substance upon which the particles fall makes but little difference in the life of the organism. On cloth a slight prolongation is occasionally noted, due perhaps to the slow drying.

VII. The mucus of the sputum exerts a destructive action on the pneumococcus.

VIII. Exposure of bacterial spray to sunlight while in suspension results in the destruction of the pneumococcus within half an hour.

IX. The conclusions of practical importance which can be drawn from the facts given in this paper are as follows:—

A. The life of the pneumococcus in moist sputum is of considerable duration, the average period being less than two weeks unless the material is exposed to direct sunlight. But as such sputum does not give off bacteria even when exposed to strong currents of air, it may be considered as innocuous except to persons handling clothes, bedding, etc., which have recently been contaminated. Under ordinary conditions, however, this sputum dries in the course of a few hours or days. The dried masses retain their virulence for a long time, and if deposited on the floor or on the bedding of the patient may be powdered mechanically, and sweeping, dusting, or brushing the contaminated articles will distribute pneumococci in the air. Fortunately, however, the organisms in the sputum do not remain long in suspension and die off rapidly under the action of light and desiccation. In sunlight or diffuse daylight the bacteria in such powder die within an hour, and in about four hours if kept in the dark. The danger of infection from powdered sputum may, therefore, be avoided by ample illumination and ventilation of the sick-room in order to destroy or dilute the bacteria, and by the avoidance of dry sweeping or dusting. Articles which may be contaminated and which cannot be cleaned by cloths dampened in a suitable disinfectant should be removed from the patient's vicinity.

B. When a person suffering from a pneumococcus infection coughs, sneezes, expectorates, or talks, particles of sputum or saliva are expelled from the mouth which may contain virulent pneumococci. Such particles remain suspended in the air for a number of hours if the ventilation of the room is good. They may be inhaled by persons in the vicinity of the patient, or they may be deposited upon various articles in the room. Whether suspended in the air or dried on surrounding objects, the writer's studies show that they become harmless in a very short time,

about an hour and a half being the extreme limit, while many of the pneumococci in the spray perish in a few minutes, especially if exposed to strong light.

In the light of these experiments the risk of infection from the pneumococcus is largely confined to those in direct contact with the person whose excreta contain the organism.

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