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THE BACTERIOLOGY OF WHOOPING- COUGH

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF
SCIENCE IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY.

(DEPARTMENT OF PATHOLOGY AND BACTERIOLOGY)

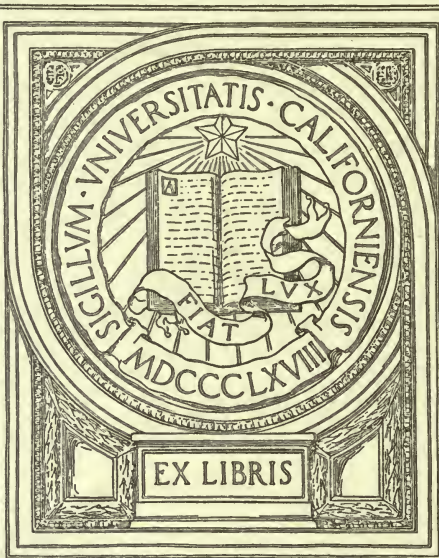
BY

DAVID J. DAVIS

CHICAGO

1906

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THE BACTERIOLOGY OF WHOOPING-COUGH*

DAVID J. DAVIS.

(From the Memorial Institute for Infectious Diseases, Chicago.)

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I. INTRODUCTION.

THE etiology of whooping-cough has not as yet been determined, notwithstanding the large number of investigations upon this subject. The disease has many serious aspects, and the problem of its etiology surely deserves to be ranked among the most important of the still numerous problems concerning infectious diseases that so far have not been solved. Like many other infectious diseases, the therapeutics of whooping-cough is in a most unsatisfactory condition; and it is likely to remain so, at least until the etiology is determined. No specific is known, and while some drugs may mitigate the symptoms slightly, none has any appreciable effect upon the general course of the disease. As regards its prevalence, it occupies a position in the first ranks of the infectious diseases of children, probably being equaled only by measles in this respect. While in strong, healthy, and older children the disease may not be considered very serious, however, owing to its prolonged course, especially in the winter months, with an abundance of serious complications and sequelæ, it must be looked upon, as the high rate of mortality abundantly proves, as one of the most dreaded diseases for the young infants and the more delicate children.

Reasons for assuming it an infectious disease.—There can be no doubt that whooping-cough is an infectious, transmissible disease. Several convincing reasons may be given for this. Transmission of the disease certainly occurs by contact between children, and Baginsky states positively that he has observed transmission by a third person. There is no doubt that the virus adheres to rooms and fomites. The frequent epidemic character of the disease, and its endemic character in cities, indicate its infectious nature. Again, the disease has a fairly definite period of incubation. There is some difference of opinion as to the length of this period, but most observers agree that it is from 7 to 14 days. Immunity is as a rule conferred by the attack. Indicative of its infectious nature is also the relatively high leucocytosis, which varies from 12,000 to 45,000, the increase in the mononuclears being particularly characteristic. Not only does the clinical course of the disease indicate an infectious catarrhal condition of the upper respiratory passages, but the pathologic anatomy substantiates such a conclusion. The mucosa, especially of the larynx and trachea,

is hyperemic and usually covered with more or less mucus. According to Arnheim, who has had numerous postmortems, there is a marked desquamative catarrh of the larynx and trachea, with swelling of the neighboring lymph glands. We know also that the posterior laryngeal wall, near the vocal cords, is the point whence severe coughing may be most readily produced, and this locality is supplied by the superior laryngeal nerve, the one which produces cough when irritated. This may be given as a reason for viewing the disease as an inflammatory irritation of the laryngeal mucosa; however, the possibility of reflex coughs due to irritation of the nasal mucosa, and the possibility of a toxic irritation of the coughing-center in the medulla, though speculative in character, might be considered.

The above facts, therefore, furnish strong evidence that the disease is due to some form of a zymotic agent, localized in all probability in the upper respiratory passages, particularly the larynx and trachea. This is important in determining the point of attack in attempting to solve the problem of etiology.

Possible nature of the virus.—From what is known concerning the nature of the causative virus of diseases of an infectious character, we may consider three possible agents, viz.: protozoa, bacteria, and enzymes. The last-mentioned agent may be dismissed by saying that, in the animal world at least, there does not exist at present evidence to indicate that such substances play any part whatever in the primary causation of any known infectious diseases. In the plant world, however, certain recently discovered facts seem to point to the possibility of enzymes being the cause of certain diseases of an infectious nature; and if this be true we should guard against overlooking such a cause in studying animal diseases of unknown etiology. Protozoa and bacteria are the common agents in the production of disease, and it is among these that we naturally look for the final solution of the problem. They may be microscopic or ultramicroscopic. We are familiar today with several diseases in animals which are undoubtedly due to ultramicroscopic organisms. Such are foot-and-mouth disease, hydrophobia, and probably hog-cholera. It has been demonstrated that these can be produced in animals, and therefore abundant material is available for careful experimental work. Whooping-cough, so far as we know, does not occur, and cannot be

produced, in animals, and we are therefore handicapped greatly in not being able to apply this method to the study of the disease. There is little doubt but that its cause, as well as that of many other human diseases of unknown origin, would be soon cleared up if only available material for experimentation could be procured. The possibility, however, of an etiologic ultramicroscopic organism should be kept in mind in studying this disease, and methods devised accordingly.

II. LITERATURE.

The literature upon the bacteriology of pertussis is as confusing as it is extensive. Almost every year one or more new organisms are described and assigned an etiologic rôle in the disease. Curiously enough, many of the investigators find organisms differing from those found by every other investigator, and their enthusiasm has not infrequently led them into controversies of a more or less personal character. After going over the literature one is clearly impressed with the idea that a great deal of rather superficial work has been done on the subject, and very little that is really thorough.

For convenience it may be well to divide the findings, as Jochmann and Krause have done, into three classes, namely protozoa, cocci, and bacilli, and discuss each briefly.

Protozoa.—Henke¹ in 1874 and Deichler² in 1886 found constantly, in the sputum of whooping-cough patients, bodies which they interpreted as protozoa. Kurloff³ in 1896 reported similar findings. From the illustrations accompanying his paper one can scarcely draw any conclusion other than that they are desquamated, ciliated epithelial cells. Behla⁴ in 1898, who also found ameba-like bodies in fresh pertussis sputum and assigned to them an etiological significance, interprets Kurloff's ciliated bodies as epithelial cells, but says that the ameba-like bodies, which Kurloff also described, were the same as his bodies. All of the above results were inadequately controlled and only indefinite data are given concerning the cases examined. The interpretations, for the most part, have been based upon observations made in unstained specimens. Behla states that staining was of no differential diagnostic value. The usual interpretation given to these results by later investigators is that leucocytes and epithelial cells have been mistaken for protozoa.

Cocci.—Certain early observations, such as those recorded by Moncorvo, Barlow and Broadbent, Haushalter and Mircoli, are of no significance, and need not be discussed. Ritter⁵ in 1892, and later in 1896, described a small diplococcus which he obtained constantly from 146 cases of pertussis, and which he considered the probable

¹ *Deutsches Arch. f. klin. Med.*, 1874, 12, p. 630.

³ *Centralbl. f. Bakt.*, 1896, 19, p. 513.

² *Baumgarten's Jahresbericht*, 1886, 2, p. 347.

⁴ *Deutsche med. Wchnschr.*, 1898, 24, p. 299

⁵ *Berl. klin. Wchnschr.*, 1892, 20, p. 1276; also 1896, 33, p. 1040.

cause. His results could not be verified by Cohn and Neumann or by Czaplewski and Hensel. In 1899 his assistant, Buttermilch,¹ attempted to identify Ritter's organism with the organism described by Vincenzi and also with that described by Czaplewski and Hensel. Neither Ritter nor Buttermilch used blood media, so that their results are not directly comparable with those obtained by later investigators.

Bacilli.—Burger² in 1883 was probably the first to call attention to numerous bacilli in the sputum of whooping-cough patients. He made no culture experiments, studying only stained preparations. He called attention to the possibility of diagnosing the disease from the very large number of organisms seen in the sputum. His results are of little value, because no cultures were made. Afanassiew,³ in 1887, described short motile, bacilli, often in masses and chains. He claims that he was able to produce the disease in dogs and rabbits by the injection of pure cultures of the bacillus. He studied 10 cases. He did not use blood media, and his method of obtaining pure cultures is questionable. Von Genser, Wendt, and Szemetschenko claim to have substantiated his results.

Spengler⁴ was apparently the first to describe an organism, in pertussis sputum, closely resembling the influenza bacillus in its morphological and biological aspects. His organisms are somewhat thicker and larger than the influenza bacillus, and have often a tendency to thread formation. Sometimes the protoplasm of the cells is filled with them. They grow only upon hemoglobin media. He considered his organism of etiological importance in the disease.

Koplik⁵ found a small, short, motile bacillus in smears and culture in the sputum in 13 out of 16 cases investigated. It grew on ordinary media, but thrived much better when hydrocele fluid was added. It was pathogenic to white mice, and intravenous injections into rabbits caused pyemia. It is very doubtful whether he was working with pure cultures, as his method of isolating the organism was hardly reliable. His results with animals suggest the presence of some of the ordinary pus producers of the mouth and throat.

Czaplewski and Hensel⁶ likewise found a small, short, polar-staining bacillus, slightly larger than the influenza bacillus, but which grew upon non-hemoglobin media. They isolated the organism from sputum on blood-serum plates. Morphologically it resembled very closely the influenza-like organisms described by Spengler, Jochmann, and Vincenzi. Buttermilch contended that it was identical with Ritter's diplococcus. Spengler said that it was the same as his bacillus, but that Czaplewski and Hensel did not obtain it in pure culture, and hence, when transferred to other media, the mixed organisms developed which were mistaken for the bacilli. Czaplewski's results were confirmed independently by Zusch,⁷ who used very much the same technique, and also by A. Cavasse. The latter,⁸ in 1904, described another organism, motile, growing only in liquids, staining very poorly, and never before observed in pertussis sputum; its significance he does not attempt to indicate. Vincenzi,⁹ in 18 cases, found a small, influenza-like organism, non-Gram staining, which grew on non-hemoglobin media. He says that it is different from Czaplewski's organism.

Elmassian¹ described an organism identical with Pfeiffer's influenza organism,

¹ *Ibid.*, 1899, 36, p. 367.

³ *St. Petersburg med. Wchnschr.*, 1887, 4, p. 349.

⁴ *Deutsche med. Wchnschr.*, 1897, 23, p. 830.

⁵ *Centralbl. f. Bakt.*, 1897, 22, p. 222.

⁶ *Deutsche med. Wchnschr.*, 1897, 23, p. 586.

² *Ibid.*, 1883, 9, p. 7.

⁷ *Centralbl. f. Bakt.*, 1898, 24, pp. 721, 769.

⁸ *Arch. gén. de Méd.*, 1904, 193, p. 1345.

⁹ *Deutsche med. Wchnschr.*, 1898, 24, p. 631.

except that it grows on serum media. He found it in eight out of 32 cases of whooping-cough, and also obtained it from cases of acute bronchitis, pulmonary tuberculosis, and pneumonia. He thinks it very probable that it is the same as Pfeiffer's bacillus, which he likewise tested, and found that it also would grow upon his serum agar. He does not consider it proved that influenza is caused by Pfeiffer's organism. Animal experiments were for the most part negative. He questioned its etiological significance for whooping-cough. There can be little doubt that this organism is the same as that described by Spengler, and later by Jochmann and Krause.

Luzzatto's *Bacillus minutissimus sputi*² corresponds to Elmassian's bacillus in practically every respect. He studied 41 cases. He also described an organism resembling the pneumococcus.

Arnheim,³ in 1900, using Czaplowski's technique, found Czaplowski and Hensel's organism both in sputum of patients and in two out of three autopsies. In 1903 he reported his findings in eight autopsies. He claims to have cultivated the organism from the lung tissue and tracheal mucus. In sections he observed the bacteria especially in the wall of the trachea, in the lungs, and in great numbers in the large desquamated epithelial cells. The organisms grow on ordinary media, and his cultures show very irregular coccus forms, thread forms, with enlarged ends, and various involution forms. With Gram's method of staining, he says that some of the organisms stain and some do not.

Jochmann and Krause,⁴ in 1901, found influenza-like bacilli in sputum of pertussis cases which belonged to three distinct classes (A, B, C) as determined by their reaction to Gram's stain and by their biological properties. In 18 out of 31 cases, among which were three autopsies, were found small, non-Gram-staining, influenza-like bacilli (Class A), which grew only in the presence of hemoglobin. This organism they called *Bacillus pertussis* Eppendorf. In four cases they found similar bacilli (Class B), which, however, grew on hemoglobin-free media. They considered this organism the same as that described by Czaplowski and Hensel, and thought, because so infrequently found, that it could not be the cause of pertussis. In three cases they found a Gram-staining bacillus (Class C) growing without the presence of hemoglobin. The occurrence of these various forms in pertussis, they think, explains the discordant results of the previous investigations. In 1903 Jochmann⁵ reports finding the *B. pertussis* Eppendorf altogether in 60 cases and in 23 autopsies. He does not report further findings of organisms belonging to the Classes B and C. He says that one has as much right to consider this bacillus the cause of whooping-cough as to consider the influenza bacillus the cause of influenza.

In 1903, Reyher⁶ reports the constant presence of Czaplowski's "Polbacterium" in 34 cases of pertussis. He found them in sputum, once in pus from the ear, sometimes in the nasal secretion, in the mucus of the larynx and trachea of nine autopsies, and in one instance saw them in sections of the larynx and trachea in the epithelial cells. He states that the organism destains by Gram. He cannot confirm Jochmann's work. His report in many respects is very indefinite.

In 1903 Neisser,¹ from conjunctivitis in measles, obtained *B. xerosis* and an influenza organism which he cultivated together for 20 generations on plain agar. He tested strains from pertussis, scarlet fever, and measles, and could not detect any difference

¹ *Ann. de l'Inst. Past.*, 1899, 13, p. 621.

² *Centralbl. f. Bakt.*, 1900, 27, p. 817.

³ *Berl. klin. Wchnschr.*, 1900, 37, p. 703; also *Virchow Archiv*, 1903, 174, p. 530.

⁴ *Ztschr., f. Hyg.*, 1901, 36, p. 193.

⁵ *Ibid.*, 1903, 44, p. 513.

⁶ *Jahrbuch f. Kinderh.*, 1903, 58, p. 605.

morphologically, culturally, or by immunization experiments. They grew well through many generations with living *B. xerosis*, but not with the dead organisms or their extracts.

Manicatide² found a short, thick, Gram-staining bacillus capable of growing on ordinary media in 67 out of 80 cases, and says it is different from any organism previously described. Animal experiments gave negative results. He immunized three sheep and two horses, and obtained a distinct agglutination at a dilution of 1:32. He treated 89 cases with the immune serum, and claims that it cures cases not over 15 days old in from 2 to 12 days. For the above reasons, he considers this germ the specific cause of the disease.

Smit³ found, in 24 cases, Jochmann's *B. pertussis* Eppendorf constantly. Neither blood serum of patients nor the serum of a horse or goats immunized to this organism had any agglutinative properties. In rabbits, by inoculation with this organism, he obtained lameness and a general infection, and also produced a conjunctivitis with the bacilli in the exudate. He treated nine patients with immunized horse serum with no perceptible effect. In 18 cases he failed to find Manicatide's bacillus in a single instance. With the bacillus which he obtained from Manicatide, he obtained some agglutination with the blood serum of pertussis patients. He does not consider either Jochmann's bacillus or Manicatide's bacillus as the cause of the disease.

Very recently Martha Wollstein⁴ isolated from 29 out of 30 cases a bacillus identical with that described by Spengler and Jochmann. This organism was agglutinated by serum of pertussis patients at dilutions of 1:200, and sometimes at 1:500. With normal serum, the tests all gave negative results above dilutions of 1:10. With influenza bacilli, the serum of pertussis patients did not react in dilutions higher than 1:20. Serum from rabbits immunized with the pertussis organism gave positive agglutination tests with the same organism at dilutions of 1:500, with the influenza organism at 1:200. Serum from animals immunized with influenza bacilli agglutinated the influenza organism at 1:200 and the pertussis organism at 1:50. Absorption tests with immunized sera also showed marked reduction in agglutinins after a saturation with the bacilli. She concludes that the pertussis organism belongs to the influenza group, but can be differentiated from *B. influenzae* by agglutination reactions with the blood of patients and immune animals. Morphologically it is slightly larger than the influenza bacillus.

Conclusions from literature.—In summing up the literature on pertussis the most striking feature is the lack of unity in the results. The work of the earlier investigators is of little value, because bacteriological technique at that time was so little developed. Undoubtedly the chief reason for the diversity of results lies in the variety of the methods employed. Lack of knowledge of the normal flora of mouth and throat also has probably had much to do with the variety of results obtained. Many of the investigators have not at all appreciated the importance of careful control of their results. These facts, together

¹ *Deutsche med. Wchnschr.*, 1903, 29, p. 462.

² *Ztschr. f. Hyg.*, 1904, 45, p. 469.

³ *Bacteriologische Onderzoekingen bij Kinkhoest*, Thesis, Amsterdam, 1905.

⁴ *Jour. of Exper. Med.*, 1905, 7, p. 335

with an evident attempt on the part of some of the investigators to exaggerate minor differences in order to be able to announce their organism as "heretofore undescribed," have been responsible for the resulting chaotic mass of data.

However, there has been found with some degree of constancy, in the sputum of patients, a small, short bacillus, often described as influenza-like, both in smears and in culture; and it may be further stated that, with few exceptions, it has been a Gram-negative organism. Further than this one finds little harmony in the results. The organism described by Spengler and by Jochmann and Krause grew only on hemoglobin media. Those described by Luzzatto and Elmassian grew in human serous fluids, and as these fluids often contain small amounts of hemoglobin, these latter organisms are probably the same as those described by Spengler and by Jochmann and Krause. Jochmann considers his organism different from that described by Spengler because of certain minor morphological differences. There is no doubt but that both are dealing with the same organism, as Spengler has very emphatically contended. The later findings of Smit and Wollstein strongly emphasize the significance of this organism. Just what organism Czaplewski and Hensel, Arnheim, Reyher, and some others, have been dealing with is very difficult to say. After reading their articles one is inclined to question, as Spengler did, whether or not they were dealing with a pure culture. Their varying reports concerning its reaction to Gram's stain, its morphology (Arnheim), and the questionable methods used in obtaining pure cultures, make one suspicious of the whole work. It is probable that they have been dealing with the same organism as that which Spengler and Jochmann described. This organism will grow, for a few generations at least, on non-hemoglobin media if another organism is present with which it may grow in symbiosis. Therefore, if sputum is smeared on such media as Czaplewski used, the Spengler-Jochmann organism will grow because there are always other organisms present. But it is a difficult matter to obtain it in pure culture, and Czaplewski and Reyher speak of the trouble they had in this respect.

No one has found an organism, either before or since Manicatide's report, that corresponds to that described by him. This is all the

more remarkable, since his bacillus grows readily on all ordinary media. He very kindly sent me a culture of his organism, which I was thus able to compare with the various organisms found in pertussis sputa of the cases reported in this paper, and in not one instance have I been able to obtain an identical organism. It is an entirely different organism from the influenza-like bacillus.

Summing up, one seems justified in concluding that, with the exception of Manicatide, probably all of the investigators, at least in more recent years, have been dealing, either in pure or in impure cultures, with the influenza-like bacillus first described by Spengler and later by Jochmann.

III. PERSONAL OBSERVATIONS.

In undertaking a study of the bacteriology of whooping-cough, my attention was naturally directed to the influenza-like bacilli. In the examinations, however, careful observations were made upon other organisms present with any degree of constancy.

Technique and material.—The sputum coughed up during a paroxysm was obtained in a sterile Petri dish and examined as soon as possible both in smear and culture. As a rule the particle of sputum was washed in several successive dishes of sterile water to remove the contaminating mouth organisms. A part of this was then used for smears and a part for culture. Two smears were made, one of which was stained with Loeffler's methylene blue, and the other with Gram's stain. As a rule I found it useful to counterstain the latter with a very dilute solution of carbolfuchsin. In this manner the Gram-staining and non-Gram-staining organisms could be very clearly differentiated, the latter, which are so numerous in pertussis sputum, taking a faint red stain, while the former appear deep blue or black.

The portion of sputum used for culture, after washing, was introduced into plain broth and thoroughly agitated; four tubes of melted agar at a temperature of 43° C., to which six drops of defibrinated blood had been added, were inoculated with varying quantities of the washed sputum and then plated. In a few of the cases sputum could not be obtained, and swabs of the throat were used. After 24 and also after 48 hours the plates were carefully examined and the various organisms present recorded.

Pigeon-blood agar was used in about one-half of the examinations. In this it was found that the influenza-like organisms, as a rule, grew more abundantly, and the colonies were larger. However, it is not essential, as they will develop on any hemoglobin media. The pigeon-blood is obtained by passing the needle of a Luer syringe into the heart of the bird at a point just above the large breast-bone under the left wing. About 5 c.c. may be obtained at one time from the heart without injuring the pigeon. This blood is at once defibrinated with a wire, and then may be used immediately, or kept in the ice-box for future use.

Bacteriology of the cases.—The sputum of 61 cases of pertussis have been studied in smear and culture. In most cases one specimen

was obtained, but in some cases several specimens were studied. In a number of the cases I examined, the sputum in hanging drop, having in mind especially the protozoa reported by several to have been found in this way. Nothing was seen which could be taken for protozoa, except occasionally a ciliated epithelial cell. The leucocytes and pus cells vary a great deal in their appearance, and one might mistake some of these for ameba. Stained preparations frequently show ciliated epithelial cells, and also numerous large, flat epithelial cells and polymorphonuclear leucocytes, but nothing that could be interpreted as protozoa was seen.

A number of specimens of whooping-cough sputum were examined with the ultramicroscope with the view of detecting the possible presence of an ultramicroscopic motile organism. This was done by diluting the sputum in broth and then passing this through a porcelain filter, which would retain all the microscopic particles. With the ultramicroscope many small particles could be seen in the filtrate but they were all non-motile, and were probably particles of proteid. Such filtrates remained permanently sterile when tested on various kinds of media.

Smears made of the sputum showed as a rule both Gram- and non-Gram-staining bacteria. The Gram-staining ones, for the most part, were lanceolate diplococci, frequently encapsulated. It is common to find these adhering to the large, flat epithelial cells so often encountered in the sputum. There is little doubt that they belong to the pneumococcus group. Only rarely is a streptococcus chain observed. Occasionally one meets Gram-positive bacilli which are shown by culture to belong usually to the diphtheria group. Cocci of the staphylococcus type are frequently seen, but are few in number. The non-Gram-staining organisms are usually most numerous, and of these a short, small bacillus, practically indistinguishable from the influenza bacillus, is most common. Sometimes it exists almost alone and in enormous numbers. It never forms threads in the sputum, and occasionally it is seen in the leucocytes in large numbers. Another non-Gram-staining organism, not infrequently met with in smear, is a biscuit-shaped diplococcus which appears to be *M. catarrhalis*. In a few of the cases, fusiform bacilli and spirilla were found in large numbers.

Upon blood-agar plates the varieties of bacteria appear so characteristic that after some experience one can differentiate them with a considerable degree of certainty. The pneumococcus, as a rule, has a typical greenish halo about the colony, and the streptococcus has usually a wide clear zone of hemolysis. Sometimes diphtheria colonies appear practically identical with the streptococcus, though as a rule the colony is whiter and more elevated and the zone of hemolysis not so wide. Not infrequently the zone is absent. The influenza-like organisms form small transparent delicate blue colonies without any zone of hemolysis. Under the microscope, they are nearly homogeneous, with a regular margin. The colonies of *M. catarrhalis* may at first be confused with the influenza-like colonies. There is no zone of hemolysis, the colony is larger, more elevated, and usually darker in color. The margin is irregular and the center is coarsely granular. Some non-hemolytic Grampositive diplococci, occasionally met with in the first 24 hours, may be mistaken for influenza-like colonies, but in smears they are readily distinguishable.

In Table 1 is given a summary of the cases of whooping-cough examined, with data indicating the more important organisms found. Under the various headings the approximate number of organisms is represented by + signs.

Of the 61 cases examined there were 33 males and 28 females; four of them were under one year of age and three were adults. The examinations were made during the fall, winter, and spring of 1904-5. At no time during this period was there an epidemic of influenza. In eight of the cases, bacilli belonging to the diphtheria group were found in the sputum. Most of these organisms had granules, and they usually produced hemolysis on the blood plates. In none of these eight cases were they numerous, and none gave a history of a recent attack of diphtheria, nor did any of them develop it later. Several of these organisms were given to Dr. Hamilton for further investigation. In one case, No. 33, the child had a well-developed, typical case of whooping-cough with no clinical evidence of diphtheria. A swab was obtained from the throat, which upon examination showed numerous influenza-like organisms, but no diphtheria bacilli, in smear or upon the blood-agar plates. Five days later the patient appeared with a well-developed membrane showing on examination many typical diphtheria bacilli, together with some influenza-like organisms.

Under the streptococcus group are included the Gram-positive cocci, whose small, delicate blue colonies have a wide, clear zone of hemolysis about them. In like manner, under the pneumococcus group are included the small colonies appearing in the blood plates, and surrounded by a green zone. While such a differentiation of

TABLE 1.
 ORGANISMS FOUND IN PERTUSSIS SPUTUM.

No.	Sex	Age	Duration of Disease When Examined	Influenza-like Bacilli	Micrococcus catarrhalis	Pneumococcus Group	Streptococcus Group	Diphtheria Group	Remarks
1.....	M	1 yr.	1 wk.	+	o	++	++	+	Spasmodic cough. Distinct whoops few days later
2.....	F	3 yrs.	5 wks.	+++	o	++	+	o	Two examinations made Whooping-cough followed diphtheria
3.....	F	1 yr.	5 "	+	o	++	++	o	
4.....	F	3 yrs.	13 "	++	o	++	+	o	
5.....	F	5 "	1 wk.	+++	o	++	+	o	Fusiform bacilli and spirilla present in large numbers. Four examinations made
6.....	M	3 "	1 "	+++	..	++	+	o	
7.....	F	4 "	6 wks.	o	++	+	++	o	
8.....	M	3 "	3 "	+++	+++	++	++	+	Fusiform bacilli and spirilla numerous in smears
9.....	M	8 "	3 "	+	o	+++	+	+	Fusiform bacilli and spirilla numerous
10.....	F	6 mo.	4 "	++	o	++	+	+	Five examinations made before finding influenza-like bacillus. It appeared during the fourth week
11.....	F	9 yrs.	4 "	+++	o	++	+	o	Three examinations made. Influenza-like organisms appeared on 15th day after first whoop noted
12.....	M	2 yrs.	5 wks.	++	o	++	+	o	
13.....	F	9 "	15 days	++	o	+++	+	+	
14.....	F	Adult	1 wk.	++	o	++	+	o	B. mucosus capsulatus present in large numbers.
15.....	F	5 yrs.	6 wks.	+++	o	++	++	o	Six examinations made. Influenza-like organisms appeared during sixth week of disease. The colonies were slightly hemolytic
16.....	F	1 yr.	3 "	+++	o	++	++	o	Sputum not obtainable. Cultures made from throat swab
17.....	F	5 yrs.	1 wk.	+++	o	++	+	o	Two weeks before child whooped examination of throat swab was negative for influenza-like organisms. At this time she had a dry cough
18.....	F	3 "	..	++	o	+++	+	o	Coughed and vomited but did not whoop until five weeks after this examination. Blood culture negative
19.....	M	3 "	5 wks.	++	+	+++	+	o	Marked tendency of influenza-like organisms to form long threads
20.....	F	6 "	5 "	++	o	+++	+	+	
21.....	F	6 "	4 "	++	o	++	+	o	
22.....	F	4 "	3 "	+	..	+	++	o	Had whooping-cough in the spring; ceased coughing during summer but began again in the fall. The examination was made at this time
23.....	F	3 "	4 "	++	..	++	++	o	
24.....	F	3 "	3 "	+	..	++	+	o	
25.....	F	2 "	2 "	+	..	++	+	o	
26.....	M	5 "	10 days	+	o	+++	o	o	
27.....	M	7 "	6 mos.	+++	o	++	+	?	
28.....	F	3 "	"	++	o	+++	o	o	
29.....	M	6 "	"	++	..	+++	+	o	
30.....	F	3 "	Several days before whooping occurred	+	..	++	o	o	Throat swab was obtained for examination several days before whooping occurred

TABLE 1—Continued.

No.	Sex	Age	Duration of Disease When Examined	Influenza-like Bacilli	Micrococcus catarrhalis	Pneumococcus Group	Streptococcus Group	Diphtheria Group	Remarks
31.....	M	Adult	1 wk.	++	..	+	++	o	Only one examination possible A second examination five days after the first showed a typical diphtheritic membrane with numerous diphtheria bacilli
32.....	F	3 yrs.	2 wks.	o	..	++	++	o	
33.....	M	6 "	4 "	++	o	++	+	..	
34.....	M	5 "	4 "	o	o	+++	+	o	Only one examination possible
35.....	M	4 "	3 "	+++	..	++	+	o	
36.....	M	4 "	4 "	+++	o	++	+	o	Obtained pure culture of influenza-like organism by having child cough against plate 10 inches away
37.....	M	4 "	3 "	+++	..	++	+	o	
38.....	F	4 "	4 "	++	o	++	+	o	
39.....	M	5 "	2 "	++++	-	++	+	o	
40.....	M	4 "	3 "	++++	o	++	+	+	Coughed severely, but first heard to whoop two days after examination
41.....	M	6 "	..	++++	o	++	+	o	
42.....	M	7 yrs.	3 wks.	++++	o	+	+	o	
43.....	F	10 "	Several weeks	++	o	++	+	o	Almost pure culture of influenza-like organisms
44.....	M	8 "	"	++	o	++	+	o	
45.....	M	9 "	4 wks.	++	o	++	+	o	Nearly pure growth of influenza-like organism
46.....	M	8 mos.	5 "	++++	o	+	o	o	
47.....	M	7 "	5 "	++++	o	+++	+	o	
48.....	M	5 yrs.	5 "	+	o	+++	+	o	Influenza-like bacilli show marked tendency to thread formation and pleomorphism
49.....	M	3 "	4 "	o	+++	+	+	o	
50.....	M	6 "	6 "	+	o	+++	+	o	Nearly recovered when examination was made
51.....	M	7 "	5 "	++	?	++	+	o	Complicated with syphilis Whooping-cough followed pneumonia
52.....	F	4 "	4 "	+	+	++	++	o	
53.....	M	17 "	4 "	+++	o	+++	++	o	
54.....	M	5 "	6 "	+	?	+++	+	o	Very severe vomiting Whooping-cough followed pneumonia and empyema
55.....	F	6 "	6 "	+	++	+++	+	o	
56.....	M	4 "	3 "	+++	o	++++	o	o	Examination by throat-swabs
57.....	M	10 "	5 "	++++	o	+	o	o	
58.....	F	8 mos.	3 "	++	o	++	+	o	
59.....	F	3 yrs.	2 "	+++	o	++	++	o	
60.....	M	2 "	3 days	+	o	+++	++	o	Blood culture was negative
61.....	M	2 "	3 "	+	++	+++	+	+	

pneumococcus and streptococcus may not be absolutely correct, for practical purposes it probably gives an approximately accurate indication of the occurrence of these organisms. As will be seen from the table, both of them are found in practically every case. Streptococci are, as a rule, not nearly so numerous as pneumococci.*

*In the pneumococcus group is included Schottmüller's *Streptococcus mitior seu viridans* inasmuch as it produces green colonies on blood-agar plates. Inulin tests of the various colonies were not made. Since these green colonies were found constantly in the throats of all cases, not only of

Organisms corresponding to *M. catarrhalis*, as described by Pfeiffer, occurred in large numbers in a few cases. They occur in pairs, being indistinguishable in smear from the meningococcus. Usually, when present, they are seen in large numbers inside the leucocytes in the sputum.

Influenza-like organisms.—In these cases attention was particularly directed to the occurrence of the influenza-like bacilli. Usually they were found in the first examination. In this series 44 of the 61 cases required but one examination to find such bacilli. In 12 of the 56 cases in which they were found, two or more examinations were necessary to find them. In five of the 61 cases they were not found, but in three of these only one examination was possible; in the remaining two, two examinations were made in one, and four in the other, with negative results. In two cases the organisms were found several days before the patients had been heard to whoop, but they had at this time a severe cough and expectorated considerable mucus. In six cases where the children were known to have been exposed to the disease, and were showing some symptoms of a dry cough, swabs of the throat were examined for these bacilli, but in none were they found. They all developed typical whooping-cough in from one to two weeks later. As to the length of time these organisms persist in the throat, present data will not suffice. One case that had developed pertussis in the spring and had ceased coughing during the summer, began to whoop again in the fall, i. e., about six months from the first attack. The sputum at this time contained an abundance of the bacilli. A brother of this child, who also had pertussis in the spring and recovered during the summer, but who did not cough in the fall, also showed large numbers of the same bacilli in his throat at this time. The organisms were as abundant in the adults as in the young children. It may therefore be said that these bacilli occur practically constantly in the throats during the spasmodic period, and even slightly before this, but probably not during the initial stage of the disease.

The bacilli were often extremely numerous, in a few cases being nearly in pure culture; this, however, depends largely on how whooping-cough, but of the diseases given in Table 2, as well as in the 20 normal throats examined, they should be considered the normal inhabitants of the throat. It is of interest that organisms of this type are practically absent on the normal nasal mucosa as shown by the examination of the same 20 normal cases that yielded them in abundance from the pharyngeal mucosa.

thoroughly the sputum is washed. In smears of the sputum they sometimes appear as the only organism present. In a few cases the leucocytes in the sputum were crowded with them, but generally they were outside the cells.

Morphology.—The organism is a small, short, non-motile bacillus, which does not take Gram's stain, does not have a capsule, and does not form spores. It stains more deeply at the ends, especially with methylene blue, and therefore may be mistaken for a small diplococcus. It stains especially well with dilute carbolfuchsin. In sputum the bacillus is nearly always single, but occasionally two or three bacilli may be seen in a chain. Their morphological characteristics in sputum are very uniform. In the culture, however, it is quite different. In many of the strains there is a marked tendency to chain and thread formation. Frequently the organisms occur as coccus forms and in thick, curved, or S-shaped threads. Sometimes threads may be seen reaching across the field of the microscope, and much thicker than the single bacillus. As was stated, they are never seen in smears direct from the throat, but may occur in the first generation in the blood plates, or develop later. They are usually more marked in a culture a few days old. When one meets these for the first time he is convinced that he has a mixed culture or some entirely different organism. These peculiar irregular forms agree well with Pfeiffer's pseudo-influenza organisms, which he isolated from cases of broncho-pneumonia. However, this fact should be emphasized, that many of the strains do not show this tendency to any extent under any circumstances, and, so far as this point is concerned, agree with Pfeiffer's description of the real influenza bacillus. As regards the size of the bacillus, the various strains all appear about the same in sputum, and, compared with the few influenza bacilli that I have been able to obtain, show practically no differences. In cultures, however, bacilli of various strains from whooping-cough vary in size so much among themselves that a comparison is difficult. Certainly many of the whooping-cough strains are as small as organisms from typical clinical cases of influenza.

Cultural characteristics.—The organisms from pertussis correspond in their cultural characteristics in every way to Pfeiffer's bacillus. It is a strict aerobe. On plates the colonies are small, moist and

dew-drop-like. They have a delicate blue color and do not hemolyze. In one case (No. 15) only, did I observe a very narrow but distinct clear zone of hemolysis about the colonies. This persisted through two generations, and then disappeared and could not be obtained again. The colonies, if not very numerous, especially on pigeon blood, will sometimes grow very large. Some have been observed over 2 mm. in diameter. As a rule they are $\frac{1}{2}$ mm. or less in diameter, and not infrequently, if numerous on the plate, require the aid of a hand lens to be seen. They do not increase in size after from 24 to 36 hours. Upon blood-agar slants they produce a delicate growth and the colonies remain discrete.

In every case, after getting the organism in pure culture on blood media, it was tested on several kinds of non-hemoglobin media, usually Loeffler's blood serum, plain agar, and milk. Many of the strains were tested on serum agar, serum broth, ascites agar, and various kinds of sugar media. In none of these did any of the organisms develop. Particular attention should be called to the fact that there is not infrequently in human serous fluids a small amount of hemoglobin, and if such fluids are used growth will occur. This fact may explain some of the reports in the literature, such as that of Elmassian, of organisms from pertussis cases growing upon serum media. To be sure of proper results, one should examine such fluids with a spectroscope before using.

Media to which yolk of egg was added did not yield any growth. Bile was added to agar, and some growth was obtained with this in the first transplantation, but continuous growth did not occur. The organism in pure culture did not grow on hematin agar. Upon CO-hemoglobin they grow as well as upon ordinary oxyhemoglobin. Inasmuch as oxygen is necessary for their growth, the CO-hemoglobin made by passing a stream of CO through blood for half an hour, was necessarily exposed to the air, and a small amount was consequently changed back to oxyhemoglobin, which might explain this growth. The abundant growth suggests, however, that the CO-hemoglobin may also be available, though it is perhaps impossible to prove this. Growth is obtained in liquid media to which a drop or two of blood is added. Plain broth plus a small amount of pigeon's blood is a good medium. The blood corpuscles soon sink to the bottom, leaving

a perfectly clear fluid above. When inoculated, this becomes turbid as a result of the growth, such a culture being very convenient for inoculations. The bacilli grow in milk, and also in the various kinds of sugar media, if a little hemoglobin is added, but no change occurs in any of them.

Only a very small amount of hemoglobin is required for the growth of these bacilli. One may dilute blood with sterile water many times, and if a few drops of this laked solution is added to agar a clear transparent medium, scarcely colored at all, is obtained, upon which the bacilli grow in abundance. This is a very convenient medium to use for their culture. The organism is rather delicate and must be transferred every five days to make sure of growth. They seem to live somewhat longer in liquid than on solid media. They are very susceptible to drying, and a temperature of 42° C. for a few hours is fatal. The bacilli have been observed through many successive generations without undergoing any essential change. Some strains have been transplanted every four or five days for six months, and their morphology and cultural characteristics remained unaltered.

These bacilli are thrown out into the air in large numbers during the coughing spell of the child, and if one is in front of the patient he is sure to inhale some of them. In five cases blood-agar plates were held in front of the patient from 6 to 12 inches away during the spasm, and in every instance the bacilli were isolated. There are always other organisms on the plate, and usually the colonies are not pure, for the bacilli are carried out from the throat in particles of spray, which one would expect to contain more than one kind of bacteria. With babies, where it is difficult or impossible to obtain sputum, examination for the bacilli may be made in this way.

Symbiosis.—Grassberger¹ has called particular attention to the occurrence of very large influenza colonies in the immediate neighborhood of colonies of other bacteria, when grown with them on blood-agar plates. He worked especially with staphylococcus, but the same effect is obtained when influenza bacilli are grown with many other varieties. He observed influenza colonies as large as 4 mm. in diameter, grown in this manner, which in pure culture are usually only $\frac{1}{2}$ mm. or less. Jochmann also observed this same

¹ *Ztschr. f. Hyg.*, 1897, 25, p. 453.

phenomenon in connection with his *B. pertussis* Eppendorf. It is well known that influenza bacilli will live and develop through several generations on non-hemoglobin media, when grown in mixed culture. Pfeiffer, before he discovered the use of hemoglobin for influenza culture, noted the growth of influenza bacilli on agar smeared with sputum, but could not successfully transplant them on this medium in pure culture. M. Neisser¹ obtained mixed cultures of *B. xerosis* and *B. influenzae* from conjunctivitis in measles, and grew them through 20 generations on plain agar. Strains of hemophilous bacilli from throats of whooping-cough, measles, and scarlet fever were in like manner carried through many generations in mixed culture. With dead *B. xerosis* or its extracts, no growth occurred. He says the presence of a living organism is necessary to produce this symbiotic effect.

Various attempts have been made to explain the mechanism of symbiosis. Ghon and Preyss² think it may be due to a reduction of the hemoglobin or hematin by some substance produced by the foreign organisms, thereby rendering the iron more available. Luerssen³ found the favorable substance residing in the cell body of the bacteria, and not in any product. He also claimed that filtrates, made after several days' growth, were favorable for the development of the influenza bacillus.

Frequently, in the blood-agar plates inoculated from the whooping-cough sputum, there was observed a conspicuous cluster of influenza-like colonies surrounding other colonies present on the plates, such as those of streptococcus, staphylococcus, and pneumococcus. They were, in this location, not only larger, but apparently more numerous, than when farther away from the foreign colonies. In order to obtain such an arrangement, it seems necessary to have a large number of influenza colonies in the plate, and a few of the foreign organisms. When the former colonies are numerous, they are often so small that a hand lens is necessary to see them; but around the foreign colonies, as a result of some favorable influence, they become much larger and are more easily visible. This appearance may be easily obtained by sowing a blood-agar plate densely with the

¹ *Deutsche med., Wchnschr.*, 1903, 29, p. 462.

³ *Ibid.*, p. 434.

² *Centralbl. f. Bakt.*, Abt. I, Orig. 1904, 35, p. 531.

pertussis bacillus, and sparsely with some other organism, such as staphylococcus. In 24 hours the symbiotic relationship is usually evident.

If pertussis sputum containing large numbers of the influenza-like bacilli, as it commonly does, is spread on plain agar or Loeffler's blood-serum, these bacilli will often grow in abundance. Other germs are always present under these circumstances, and undoubtedly their growth has much to do in causing the influenza-like bacilli to develop on the hemoglobin-free media; for, in sterile sputum alone, the latter do not grow. If transferred thus in impure culture upon plain media, they often survive several generations, and their growth may be easily determined by making blood-agar plates at the time of each transplantation. It was practically this technique that Czaplewski and Hensel, and others who obtained similar results later, used to isolate their bacillus, and one is strongly tempted to believe that they have thus been cultivating in impure culture the same hemophilous bacillus found by Spengler and Jochmann. As already stated, this was Spengler's explanation of their results. Such an interpretation seems far more reasonable than the possibility that Czaplewski's organism, found as frequently as he reported, was an entirely different organism from the hemophilous bacillus found by so many.

The growth of the several strains of influenza-like bacilli from pertussis was tested upon various media, to which were added dead bacteria (staphylococci, streptococci) and filtrates of cultures; the results were uniformly negative. All of the experiments performed indicated that the symbiosis is dependent on the presence of the living organisms.

The symbiotic relation of other bacteria to the influenza-like organisms is shown by the increased pathogenicity of the latter for animals. This will be more fully discussed under the head of animal experiments.

IV. ANIMAL EXPERIMENTS.

Literature.—The results obtained by the inoculation of whooping-cough virus into animals by various investigators have for the most part been negative. The older reports of producing the disease in dogs can hardly be considered seriously. Afanassiew reported that

he produced a real whooping-cough in animals by the inoculation of his organism, but these results have never been substantiated. Ritter's animal experiments were not conclusive. Injected into the circulation, his diplococcus produced no effect. Czaplewski and Hensel, and also Zusch, obtained no results in animals with their organism. Koplik reported that intravenous inoculation with his bacillus produced suppurative arthritis, that 5 c.c. of culture inoculated into white mice killed in one week, and that the organisms were present in the blood; but that inoculation of guinea-pigs and rabbits with sputum gave negative results. Vincenzi's results were negative. Elmassian, with his organism, produced death in young guinea-pigs by intraperitoneal inoculation in 24 hours or less, and isolated the bacillus from the fluids and heart's blood; there was marked phagocytosis. Intravenous injection into rabbits produced some cachexia, while pigeons and mice did not react. Manicatide obtained no results by inoculating his bacillus "z" into the nose and trachea of guinea-pigs, cats, dogs, and monkeys. Jochmann and Krause were not able to produce any effect either with sputum or pure cultures of their bacillus, when inoculated into the nasal cavities, throat, or peritoneum of animals. On the other hand, Smit found Jochmann's bacillus pathogenic for young guinea-pigs by peritoneal injections, and that it also produced lameness, general infection, and death in rabbits by intravenous injection. He also produced double-sided conjunctivitis in a rabbit, and obtained the bacillus in the exudate. A distinct reaction occurred in the horse when injections were made with the bacillus for immunizing purposes. Wollstein reports negative results with the influenza-like organism from intraperitoneal and subcutaneous inoculations in white mice, guinea-pigs, and rabbits, and also from a subdural inoculation in a rabbit.

For comparison with the above results, we may briefly refer to results obtained with Pfeiffer's bacillus. Inoculation experiments with the sputum of influenza patients and with pure cultures of the bacillus upon guinea-pigs, rats, mice, and doves are generally without result. In rabbits and monkeys some symptoms have been produced. Pfeiffer, by injecting the bacilli into the lungs of monkeys, observed symptoms simulating those of influenza, and by intratracheal injection of the bacilli killed a monkey in eight hours. There being no

anatomical change visible, this latter result was attributed to the toxic products—surely a most doubtful conclusion. In rabbits, by intravenous inoculation of living bacilli, dyspnea and marked weakness were produced, which Pfeiffer also attributes to toxins, because the same result was produced with dead bacilli. Kolle and Delius substantiated these results, and also showed that influenza bacilli would develop in the peritoneal cavity of animals, especially guinea-pigs. The exudate contained many bacilli, partly free and partly in the cells. They were able to raise the virulence of the bacilli by successive animal inoculations, and in filtrates of cultures obtained a specific but very labile poison. Jacobson¹ increased the virulence of the influenza organism by simultaneous injection with streptococci, and Slatineano² raised the virulence by injecting lactic acid at first and one-half hour later the influenza bacilli.

Experiments on lower animals.—The influenza-like bacilli isolated from the whooping-cough patients in this series showed on the whole a low degree of virulence for animals; however, some very definite results were obtained. Guinea-pigs were found most satisfactory for this purpose. White rats are susceptible, but less so than guinea-pigs; rabbits, especially young ones, are also susceptible when inoculated intraperitoneally. But few inoculations were made in these animals, however. Two blood-agar cultures inoculated intraperitoneally into guinea-pigs will rarely kill in 24 hours. Usually the animal is sick the next day after inoculation, but will gradually recover. If the amount is increased, death will usually occur in 24 hours or less, and from the peritoneal cavity, pleural cavity, and heart's blood, the bacilli are obtained in abundance. In the blood the bacilli are so numerous at times that they may be found without great difficulty in smear. A few drops, allowed to run over the surface of an agar slant, usually shows an abundance of colonies in 24 hours. Blood removed from the heart before death also shows the presence of bacilli, so that their occurrence is not due to a postmortem invasion, but there seems to be an actual multiplication of them in the living blood.

When the animal dies in 24 hours or thereabouts after inoculation into the peritoneal cavity, the peritoneal and pleural exudates are clear, serous in character, and soon clot on standing. The bacilli

¹ *Archiv de méd. expér.*, 1901, 13, p. 425.

² *Comp. rend. de la Soc. de Biol.*, 1901, 51, p. 850

are very numerous. But few polynuclear leucocytes are present, and these are usually filled with the bacteria. If the exudate of an animal inoculated with an amount insufficient to kill in 24 hours is examined about the second or third day, a very different picture is presented. The exudate is purulent and contains flakes of fibrin. The polynuclear leucocytes are very abundant, and are busily engaged ingesting the bacteria. There are also present, in less numbers, large mononuclear cells. These cells often contain one or more polynuclear leucocytes filled with bacilli. The ingested leucocytes may be seen in various stages of intracellular digestion, some being nearly perfect, while others show nothing but fragments of the nucleus.

A number of animal experiments were made to determine the effect of introducing another organism with the influenza-like bacilli into the peritoneal cavity. This had been done with the influenza bacillus and the streptococcus by Jacobson, who found that the virulence of the bacilli could thus be increased so that much smaller doses of the bacilli alone would be sufficient to kill. He obtained both the bacilli and streptococci in the body fluids and heart's blood. In my experiments a non-virulent *Staphylococcus pyogenes aureus* was chiefly used. Two c.c. of a broth culture of this organism produced no effect when inoculated into the peritoneal cavity of guinea-pigs. When $\frac{1}{2}$ c.c. of this culture was inoculated with two blood-agar slants of the pertussis organism, which, as above stated, rarely killed, death invariably followed in 24 hours or less. From the peritoneal exudate both organisms were isolated; from the heart's blood, however, as a rule only the influenza-like bacilli were obtained, and no staphylococci. In two animals, where large amounts of staphylococci were injected, a few were obtained also in the heart's blood. After passing the bacilli in symbiosis with staphylococci through six animals, one blood-agar slant alone would kill an animal in 24 hours, i. e., its virulence had been more than doubled. The effect of *M catarrhalis* and an avirulent streptococcus upon the influenza-like bacilli was found to be very similar to that of staphylococcus, i. e., the bacilli were distinctly more virulent when associated with these organisms than when inoculated alone. In all these experiments controls were made by inoculating animals with the same or larger amounts of the organisms separately.

The increased pathogenicity of this organism when growing with another organism is of great importance, and deserves much more attention and study than has been given to it. This property is directly associated with the other symbiotic phenomena noted earlier in the paper, and probably depends upon the same factors. Under the conditions in which we find these bacilli in the throat, for instance, they are always associated with other organisms, and therefore are thriving under circumstances which permit a manifestation of their greatest virulence and most luxuriant growth. It is also quite possible that the injurious products of such growth may be not only more abundant, but even of a different and more toxic character. It would therefore be improper to draw conclusions from data obtained by growing the organisms in pure culture.

If one injects intraperitoneally two or three drops of lactic acid in 2 c.c. of water one-half hour before the injection of influenza-like bacilli from pertussis, the resistance of the animal toward them is diminished, and it will therefore succumb to smaller amounts of bacilli than otherwise. This is due, in all probability, to the effect of the acid upon the leucocytes, rendering them unable successfully to combat the bacilli. This corresponds with the results obtained by Slatineano, who used this method to raise the virulence of influenza bacilli.

Sputum from whooping-cough patients, containing large numbers of the bacilli, and also pure cultures of this organism, were inoculated into the throat and nasal cavities of a monkey, with no effect. Another monkey, inoculated in the same way with influenza bacilli from a typical case of acute influenza, likewise showed no reaction.

The reported attempts to produce whooping-cough in human beings by inoculations are of little significance. After producing pertussis-like symptoms in a rabbit with a fungus isolated from pertussis sputum, decaying oranges, apples, etc., which he considered the cause of the disease, Tschamer¹ and his assistant inhaled some of the pulverized mold. In eight days they had symptoms of spasmodic coughing, and the fungus was found in the sputum. In this manner the etiology was settled. Ritter² and his assistants inhaled cultures of his diplococcus with no result other than a slight mechani-

¹ *Jahrbuch f. Kinderh.*, 1876, 10, p. 174.

² *Berl. klin. Wchnschr.*, 1893, 30, p. 1154.

cal irritation. Czaplewski states that, while working with his organism, he was taken with a coryza accompanied with some cough, which lasted for about a week. He obtained his bacillus from his nose and throat in large numbers.

Human inoculation.—As no attempts are on record to inoculate man directly with the influenza-like bacillus from pertussis, it seemed desirable to make such a test with the view of determining its possible pathogenicity. A young man, who, according to his statement and also that of his relatives, had never had whooping-cough, agreed to submit to inoculation with hemophilous organisms isolated from the pertussis cases. This was done with his full consent, and he was informed concerning the character of the material inoculated and the possible results anticipated. He was healthy in every way, and an examination of his throat made previous to the inoculation showed only the usual bacterial flora of the normal throat. There were no influenza-like organisms present. Pure blood-agar cultures, 24 hours old, of influenza-like bacilli, isolated from an uncomplicated case of whooping-cough, were smeared upon the nasal mucosa and the tonsils. On the second day, almost exactly 48 hours after the inoculation, the patient complained of a chilly sensation, a cold sweat, some headache and weakness. His temperature rose from normal (98.4° F. the day previous) to 100.2° F. The same evening it registered 100.1° F. The next morning it was normal again, but rose during the day to 99.5°. The third day his temperature was practically normal and continued so from that time on. He complained somewhat of headache and of feeling unwell, and on the second morning said he coughed a little. His throat, upon examination when the first symptoms appeared, was slightly hyperemic; the next morning there was present a thick layer of mucus upon the pharyngeal wall, and from this time on he coughed, or rather hacked up large quantities of a stringy, tenacious mucus. This condition continued, becoming gradually less marked, and was still apparent at the end of four weeks. After the first few days he felt perfectly well, complaining only of slight discomfort in the throat and the light cough. This cough was not spasmodic and did not resemble whooping-cough. The leucocyte count on the second day was 9,200.

Bacteriological examination of the mucus from the throat, obtained

on the first day of symptoms revealed almost a pure culture of the influenza-like bacilli. They were present both in the throat and on the nasal mucosa, in enormous numbers. Direct smears of the expectorated mucus showed practically no other organism. They were identical in every detail with the organisms inoculated. Examinations of the throat were made every few days for four weeks, after which time the patient was not accessible. The number of organisms present became gradually less, but at the end of that time they were still present in considerable numbers.

From this experiment we must conclude that this organism develops in the human throat when implanted there, and is capable of causing a distinct reaction. The fact that pertussis was not produced by this one inoculation by no means excludes this organism as an etiologic factor in the disease. For, in a case of this kind, it is impossible to be absolutely sure that the individual has never had the disease, and adults at this age may be immune. The result does indicate surely that this organism, present almost constantly as it is in pertussis, and capable of producing such reaction in an adult, must be of some significance in the disease, and is at least not always a harmless saprophyte.

V. INFLUENZA-LIKE ORGANISMS IN OTHER DISEASES AND IN NORMAL THROATS.

The occurrence in other diseases of what appears to be the same organism, morphologically and culturally, is of interest. A number of examinations were made of sputum and throat swab by the same method as that used in whooping-cough, from cases of measles, acute influenza, epidemic cerebrospinal meningitis, bronchitis, varicella, and from normal throats.

Measles.—Twenty-two cases of uncomplicated measles were examined. The sputum was obtained in most of the cases, but in a few this was impossible, and throat swabs were used. All were examined during the stage of eruption, and most of them had at the time the usual cough present early in the disease. Only one examination was made in each case. The influenza-like bacilli were isolated in pure culture in 13 of the 22 cases. In four of the cases they were the predominating organism; in some cases they were very few

in number. Streptococci and pneumococci were found in every plate, the latter always predominating. The *M. catarrhalis* was found in 10 of the cases, being extremely abundant in two. Diphtheria bacilli were obtained from the throats in two cases. They were large curved bacilli containing large granules, and the colonies showed a distinct, clear zone of hemolysis. One of the cases had a profuse nasal discharge during convalescence. In this material were found by culture pneumococci, streptococci, a few diphtheria bacilli and a considerable number of influenza-like bacilli. Four of the cases were complicated with otitis media, and the pus was examined in each case. In one case the influenza-like bacilli were obtained nearly pure, there being present also a few streptococci. In this case the bacilli had been obtained from the throat about a week previously; 11 days later the pus was again examined, and showed no influenza bacilli, but nearly a pure culture of a bacillus of the diphtheria group. From the other three cases streptococci were obtained, pure in one of them, and streptococci and diphtheria bacilli, mixed, in the remaining two.

Clinical influenza or grippe.—The sputum was examined from 17 cases that were diagnosed clinically as acute influenza. They occurred at a time when there was reported to be an epidemic of grippe in the city. In only three cases were the influenza bacilli found, and in only one of these were they very numerous. Streptococci were very conspicuous in these cases, and while not so numerous as pneumococci, they were more abundant than in the other groups examined.

Epidemic cerebrospinal meningitis.—In five cases of typical cerebrospinal meningitis the influenza-like bacilli were found either in the nose or throat of four. The case in which it was not found had been sick for three weeks when examined, and at that time showed no nasal or throat symptoms. The other four cases were examined early in the disease, and showed some nasal and throat symptoms. In one of these the meningococcus was isolated from the nasal cavity and also the sputum, and in this same case the influenza-like bacilli were also present in large numbers, being by far the predominating organism. The meningococcus was cultivated from the cerebrospinal fluid in each of the five cases.

Bronchitis.—This comprises a more or less miscellaneous group of cases. All had bronchitis, some for a long period, while in others it occurred in a more acute condition. In some cases it was a complication of some other disease. The influenza bacilli were found in five of the 12 cases examined. In two of these cases they were very numerous, by far exceeding all other organisms present. One case in this series was a typhoid which began with a marked bronchitis. Here was found, in practically pure culture, the *M. catarrhalis*, but no influenza-like organisms. This micrococcus was found in only one other case of this group.

Varicella.—Throat swabs from 11 cases of varicella in young children were examined in the usual way. All of the children had a slight cough, and three had at the time of the examination a gonorrhoeal vaginitis. The influenza-like bacilli were isolated in seven of the eleven cases; in two they were numerous. Streptococci were found in eight, diphtheria bacilli in one, and the *M. catarrhalis* in all of the cases. The latter organism was very numerous in two cases in which the influenza-like organisms were absent.

Normal throats.—For these examinations swabs were made from the posterior wall of the pharynx and were for the most part obtained from medical students. In 20 cases the influenza-like bacilli were found twice; in neither case were they numerous. The throats of these two individuals appeared normal, and they gave no history of any throat trouble for at least three months previously, remaining perfectly well for several weeks at least following the examination.

Table 2 gives a summary of the occurrence of influenza-like bacilli in the diseases investigated. It should be noted that in all the diseases except whooping-cough but one examination was made. In this disease, in a number of cases, several examinations were made before finding the bacilli. It is therefore quite probable that if more examinations were made in the other conditions the percentage of positive results would have been higher.

The bacilli from these various sources were carefully observed and their properties tested. Every organism was transplanted to non-hemoglobin media, and not one was found which would grow. Particular attention was given to the test on ascites-agar and serum-agar, as so many reports have been made that organisms of this kind

TABLE 2.
OCCURRENCE OF INFLUENZA-LIKE BACILLI IN VARIOUS DISEASES AND IN NORMAL THROATS

	No. of Cases Examined	Influenza-like Bacilli Present	Influenza-like Bacilli Not Found	Percentage of Positive Findings
Whooping-cough.....	61	56	5	92
Epidemic cerebro-spinal meningitis....	5	4	1	80
Varicella.....	11	7	5	63
Measles.....	22	13	9	59
Bronchitis.....	12	5	7	42
Influenza (grippe).....	17	3	14	18
Normal.....	20	2	18	10

occur which will develop upon these media. In no instance did a continuous growth occur when proper precautions were taken to use fluids free from hemoglobin. Their cultural characteristics were identical, and morphologically there were no constant differences between them. In every group strains were found with a tendency to marked thread formation, and there was also considerable variation in the size of the various strains, but nothing was observed that would characterize any particular group. Animal experiments showed that the bacilli from the different groups possessed about the same pathogenic power as those isolated from whooping-cough cases. Even those isolated from normal throats manifested the same low degree of virulence as the other strains. A large quantity of the bacilli is necessary to produce death, and the symptoms are not those of a toxemia but of a general invasion, with numerous bacilli in the blood.

Literature.—In the literature there are numerous references to the occurrence of hemophilous bacilli in many infectious diseases and diseases of the respiratory tract. They have usually been referred to as influenza bacilli (Pfeiffer's bacillus), occasionally as pseudo-influenza organisms, and for the most part have been looked upon as secondary invaders, except in influenza, in which the hemophilous bacilli have pretty generally come to be considered as the specific organism. Pfeiffer, in 1893, in his classical work on influenza, was the first to announce this, and since then an enormous mass of literature has accumulated, most of which tends to substantiate the idea that his bacillus is the specific cause of this disease. If the evidence is carefully examined, however, it is found

that in reality the specificity of this organism does not rest upon any too sure foundation. It does not meet Koch's requisites. In many epidemics the bacilli are constant in the throats, being often present in large numbers; and they have been obtained from abscesses and numerous other lesions in the body. In postmortems the organisms are found in large numbers, especially in the respiratory passages and the lungs. It is somewhat questionable whether the influenza bacillus has ever been obtained from the blood during life. Canon's results are absolutely discredited by Pfeiffer himself, and the work of Jehle, Isambert, Rosenthal, and others needs further confirmation. The pathogenicity of the bacilli for animals is very low, and the experiments of Pfeiffer in regard to the presence of toxins are not at all convincing—though the more recent work of Kolle and Delius and Slatineano indicate their occurrence. These facts are hardly sufficient to demonstrate beyond question that the organism is specific, and that it is not merely a secondary invader.

It is interesting that the French especially have always been skeptical about the etiologic rôle of Pfeiffer's bacillus in influenza; and many data have been presented in opposition to the view that it is specific for this disease. Elmassian,¹ in 1899, could not distinguish between organisms obtained from whooping-cough, acute bronchitis, pulmonary tuberculosis, and pneumonia. He considered them all identical with Pfeiffer's bacillus of influenza, and said that it was not proved that this bacillus was the specific cause of grippe, but should be considered only the probable cause. Rosenthal² quotes Metchnikoff as saying that Pfeiffer could not find the influenza organisms in an epidemic of grippe in 1899. He thinks it an ordinary microbe of the pathologic flora of the lungs, and not specific for grippe. Sacquépée³ studied a typical and very severe epidemic of grippe in a garrison, and found Pfeiffer's bacillus in only the latter part of the epidemic (February). At other times he found a Gram-negative, motile, typhoid-like bacillus, and also pneumococci and streptococci. He thinks that the influenza bacillus is not specific, and that grippe may not be due to any specific microbe. Bezançon and Israëls de Jong,⁴ in an epidemic of grippe, studied the expectorations of 25 cases bacteriologically, and found Pfeiffer's bacillus to be very rare. They call special attention to "*Micrococcus catarrhalis*" and to "*paratetrage zoogleique*" which were very common. They found pneumococci, streptococci, pseudo-diphtheria, pneumobacilli, and rarely staphylococci. They conclude that grippe is not caused by Pfeiffer's bacillus, but is due to a variety of organisms of exalted virulence. Kleinenberger,⁵ in 27 cases of epidemic influenza, found the influenza bacillus only eight times. The *M. catarrhalis* was present in nearly every case. Lord,⁶ of Boston, has

¹ *Ann. de l'Inst. Past.*, 1899, 13, p. 621.

² *Compt. rend. de la Soc. de Biol.*, 1900, 52, p. 266.

³ *Arch. de méd. expér.*, 1901, 13, p. 562.

⁴ Reviewed, *Bull. de l'Inst. Past.*, 1905, 3, p. 372.

⁵ *Deutsche med. Wchnschr.*, 1905, 31, p. 575.

⁶ *Boston Med. and Surg. Jour.*, 1905, 152, pp. 537 and 574.

studied non-epidemic infections of the respiratory tract, and has clearly shown the similarity, both clinically and pathologically, of the infections due to influenza bacilli and other organisms, such as pneumococcus and *M. catarrhalis*. Von Jaksch¹ has described a condition, under the name of pseudo-influenza, which is indistinguishable clinically from influenza, but which is not caused by the influenza bacillus. He concluded that it was due often to a streptococcus invasion, because of the large number of these organisms found; in some the cause could not be determined, but he was sure it was not due to influenza infection.²

The results of these investigations correspond very well with the findings in the series of influenza cases reported in this paper. While it is undoubtedly true that in many epidemics of influenza Pfeiffer's bacillus exists constantly in the nasal and oral excretions in very large numbers, and to the exclusion largely of other bacteria, and that its presence corresponds with the course of the disease, it is equally true that there occur epidemics, and also sporadic cases, of a condition which appears to be identical with influenza clinically, but which shows Pfeiffer's bacillus only occasionally, and other organisms, such as pneumococci, staphylococci, and *M. catarrhalis*, very commonly and in large numbers. The most rational interpretation of these facts seems to be that there are a number of organisms which may give clinically the same picture, and which may exist in the respiratory passages either in nearly pure culture; or they may occur together.

The fact that many cases, indistinguishable clinically from acute influenza, occur both in sporadic and epidemic form not associated with the influenza bacillus, should be more widely known among the medical profession at large. For ever since the great epidemic of 1889-90, almost everything bearing any resemblance at all to influenza is assumed, without a bacteriologic examination, to be an influenza infection. Attention should be called also to the fact that smears of oral and nasal excretions are very unreliable in the diagnosis of this disease, and that, to be sure of an influenza infection, cultures on blood-agar plates should be made. Clinical data, there-

¹ *Berl. klin. Wchnschr.*, 1899, 36, p. 425.

² Since this paper was written an article upon influenza infections by Jochmann (*Deutsches Arch f. klin. Med.*, 1905, 84, p. 470) has appeared. In 36 cases of epidemic influenzas he was able to find influenza bacilli in only 13. Pneumococci and streptococci were very common. He thinks that the clinical picture of acute influenza may be produced by organisms other than the influenza bacilli; especially the pneumococcus, streptococcus, and perhaps the *M. catarrhalis*. He discusses the occurrences of influenza-like bacilli in the infectious diseases and says the organisms from whooping-cough are indistinguishable morphologically and biologically from the bacillus of Pfeiffer. His results agree very well with those given in this paper.

fore, unless accompanied by careful bacteriological examinations, are of little value. This is mentioned because in the literature, even of late years, so much is written upon influenza without definite knowledge of the presence of influenza bacilli.

Besides influenza, in which hemophilous bacilli occur, and are the probable, but not the demonstrated, cause of the disease, there are numerous other diseases in which hemophilous bacilli, usually called influenza or pseudo-influenza organisms, have been frequently found. A brief résumé of the more important literature upon this point will here be given.

Pfeiffer, as already stated, found what he called pseudo-influenza bacilli in broncho-pneumonia, and distinguished them from influenza by their greater size and their tendency to form threads. Numerous writers since that time have noted the marked tendency to form threads in bacilli from cases of influenza, and have also noted considerable variation in size in various strains of such organisms. Also, attention has been called to the occurrence in other conditions of bacilli not differing morphologically from Pfeiffer's influenza bacillus. Pfeiffer's distinction, therefore, between the bacilli from influenza cases and those from other sources does not appear to hold true, and the pseudo-influenza, for the present, must be considered identical with the influenza bacillus. Elmassian found influenza bacilli in acute bronchitis, tuberculosis, and pneumonia. Rosenthal¹ found them in 15 out of 19 cases of broncho-pneumonia, nearly pure in two. Susswein² obtained them in 10 out of 21 cases of measles. Lo'd,³ in a very valuable paper, reports his findings in diseases of the respiratory tract, chiefly acute and chronic bronchitis. He obtained influenza bacilli in every way corresponding to Pfeiffer's bacillus in 56 out of 186 cases (30 per cent), and in 47 cases the bacilli were in overwhelming numbers. These cases were examined at a time when there was prevalent no epidemic of any acute respiratory disease. Smith⁴ found influenza bacilli five times in 73 cases of lobar pneumonia. Leiner⁵ obtained them constantly from the bronchial secretion and lungs of 11 postmortem cases of diphtheria. Neisser⁶ found them in cases of measles, scarlet fever, and whooping-cough. Jehle⁷ found influenza bacilli in postmortems as follows: In 48 cases of scarlatina, 19 times in the lungs, six times in the tonsils, and 22 times in the blood; in nine cases of varicella, nine times in the lungs and five times in the blood; in 24 cases of pertussis, 24 times in the lungs and 12 times in the blood; in 15 cases of diphtheria, nine times in the lungs and five times in the blood. Liebscher,⁸ in 57 cases of measles, found influenza bacilli 11 times, and in 60 cases of scarlatina found them three times. He examined only the nasal secretions. Auerbach⁹ found, in swabs from the tonsils and larynx of 700 cases of acute infectious diseases, the influenza bacillus 12 times in diphtheria, three times in scarlatina, six times in what he calls diphtheria-scarlatina, seven times in diphtheria-morbilli, and 10 times in anginas. Kleinenberger¹⁰ found hemophilous

¹ *Compt. rend. de la Soc. Biol.*, 1900, 52, p. 226.

² *Wiener klin. Wchnschr.*, 1901, 14, p. 1149.

³ *Brit. Med. and Surg. Jour.*, 1905, 152, p. 537.

⁴ *Jour. Boston Soc. of Med. Sc.*, 3, p. 274.

⁵ *Wiener klin. Wchnschr.*, 1901, 14, p. 1001.

⁶ *Deutsche med. Wchnschr.*, 1903, 29, p. 462.

⁷ *Ztschr. f. Heilkunde*, 1901, 22, p. 190.

⁸ *Prager med. Wchnschr.*, 1903, 28, p. 99.

⁹ *Ztschr. f. Hyg.*, 1905, 47, p. 259.

¹⁰ *Deutsche med. Wchnschr.*, 1905, 31, p. 575

bacilli in 18 out of 25 cases of pertussis, upon the conjunctiva in three out of nine cases of measles, once in the heart's blood in measles postmortem, once in scarlet fever and once in a case of urethritis. So far as I have been able to determine, there are no reports in the literature upon the occurrence of influenza bacilli in normal throats.

To sum up the literature, the results agree very well with the data given in Table 2, so far as they are comparable. Süsswein's results in measles and Lord's results in bronchitis correspond very closely with those in the table. The findings in influenza agree in general with those obtained by many investigators, especially the French. I do not know of any positive findings in the literature of influenza bacilli in the nose or throats of cases of epidemic meningitis. All the results taken together indicate that we have in these bacilli an organism occurring very commonly in a large number of diseases, and undoubtedly present, at times at least, in all inflammatory conditions of the respiratory passages whatever their character may be. The evidence is conclusive that this organism at times exists as a saprophyte, as indicated by its appearance in normal throats, and that it is also capable of causing inflammatory changes, as shown both by human and by animal experiments, and by the vast amount of pathologic data. The all-important question is, whether we are dealing in all these various conditions with the same organism, varying only in its degree of virulence, or whether we have to do here with a group of very closely related organisms. Morphologically, tinctorially, and culturally, from the results obtained from the various strains isolated in the cases here reported, and in the cases reported in the literature, we must conclude that we have no means of distinguishing them. Reasoning from analogy, we should expect a group, as occurs with so many of the other bacteria; and the chief problem now before us is the careful application of every possible means, particularly in the way of biological experiments, to differentiate the organisms. Already considerable evidence from this point of view exists, but it is conflicting. Wollstein's experiments upon agglutination with the organisms from influenza and pertussis are extremely important and suggestive. They are in accord with results obtained by Cantani¹ with the influenza bacillus, but are opposed, directly or indirectly, to the results obtained by several investigators (Neisser, Smit, Lord, Jehle, Meunier).

¹ *Ztschr. f. Hyg.*, 1903, 42, p. 505.

It is perhaps worth while at this time to call attention to the fact already noted by Jochmann, that whatever may be said in favor of the influenza bacillus as the cause of influenza, practically the same thing may be said in favor of the organism isolated from the cases of pertussis as the cause of that disease. The constant occurrence of the organism at the apparent seat of disease in practically all uncomplicated cases, the parallelism between the general course of the disease and the relative abundance of the organisms, the presence of the organisms in the lungs and various secretions in postmortems, the low pathogenicity of the organism for animals—all these hold true for one equally as well as for the other. The medical world, with few exceptions, has come to recognize the influenza organism as specific for this disease, but it surely is not yet ready to accept the cause of whooping-cough as settled. This fact may be interpreted in two ways; it may be used to indicate upon how insecure a foundation the specificity of the influenza bacillus rests, or it may be used as an argument in favor of the specificity of the pertussis organism. Evidently, with the present data at hand, it is impossible to settle either question absolutely.

Pertussoid, pseudo-pertussis, etc.—A few words may be said concerning a condition variously referred to in the literature as a whooping-cough-like cough in influenza, pertussoid, pseudo-pertussis, etc., by Leichtenstern, Forchheimer, Filatow, Guidi, Pestalozza and others. From the description given, it would appear that these conditions are intermediary forms between influenza on the one hand, and whooping-cough on the other. This is certainly suggestive in view of the fact that bacilli so nearly alike, if not identical, are found so constantly in both these diseases. Forchheimer¹ was able to find the influenza bacillus in but few of the cases, but found streptococci constantly.

I have had an opportunity of examining only two cases which might come under this class. Both were adults, and both had come in contact more or less with whooping-cough patients. Each had a very severe cough, lasting several weeks, spasmodic in character but without distinct whoops. In the sputum of both, influenza-like bacilli were found, quite numerous in one, but very few in the other.

¹ *Archives of Pediatrics*, 1900, 17, p. 801.

In the former were also many streptococci, while in the latter there was nearly a pure culture of pneumococcus. A careful study, with particular reference to the influenza-like bacilli, should be made of the flora of the oral and nasal secretions of a large series of such cases, and especially those cases of cough occurring in parents or persons associated with children having pertussis, and often referred to as a sympathetic cough, and the like.

VI. NOMENCLATURE OF THE BACILLI.

Before concluding, it may be well to discuss briefly the nomenclature of the bacilli of this class. As a general term for organisms of this type, which only grow upon hemoglobin media, "*Bacillus hemophilicus*," used so generally by the French, certainly seems appropriate, inasmuch as it describes their most important characteristic. Such a term would include Pfeiffer's bacillus, the pseudo-influenza bacillus, all the bacilli described as influenza-like or as influenza bacilli found in whooping-cough, measles, scarlet fever, diphtheria, pharyngitis, varicella, bronchitis, tuberculosis, etc., Müller's trachoma bacillus, and a few other organisms having this property and isolated from various sources. Should differences be detected between the various organisms, as in all probability there will be, at least between some of them, special names may then be assigned them; this has been done, by those who believe that there are detectable differences, with organisms from influenza and whooping-cough. As for the last-mentioned organism, if the names of individuals are used to designate it, it should be known either as Spengler's bacillus or the Spengler-Jochmann bacillus. After reading Spengler's description of this organism, it is difficult to see how anyone can believe that he was dealing with an organism different from that described by Jochmann, as the latter writer contends. Spengler called this organism the pertussis bacillus, and if this organism is proved beyond question to be specific for the disease and different from *B. influenzae*, this term should be used, and Jochmann's term, "*Bacillus pertussis* Eppendorf," discarded. However, until these organisms are definitely distinguished from each other, it would seem that the special term, *Bacillus influenzae*, should be used for all.

VII. SUMMARY AND CONCLUSIONS.

1. In the sputum of pertussis patients there is, almost constantly, an organism which, morphologically and culturally, is identical with the influenza bacillus. It is usually in greater abundance than any other organism of the sputum.

2. This organism is most abundant during the spasmodic stage of the disease. It has been found several days before the whoops began, and as long as six months after the disease.

3. During the coughing spasms it is thrown out in the surrounding air in large numbers.

4. Alone, its pathogenicity for animals is low; when associated with other organisms, this property is decidedly increased.

5. When transplanted in the human throat, this organism grows in abundance and gives rise to a distinct reaction. It is therefore not a harmless saprophyte.

6. Symbiotic phenomena are manifested by this organism exactly as by Pfeiffer's bacillus.

7. Organisms which have not been differentiated from this bacillus occur in a variety of throat affections, and occasionally in normal throats.

8. The evidence at hand will not permit a definite statement for or against the specificity of this organism for whooping-cough.

9. Granting that it is not specific, its significance as a harmful secondary invader cannot be questioned.

10. This organism was described first by Spengler in 1897, and later by Jochmann, Krause, and others. Undoubtedly many have observed it, but failed to isolate or describe it properly.

I wish to acknowledge my indebtedness to Dr. Hektoen for suggestions and to Drs. Baum and Weaver, attending physicians at Cook County Hospital, for the privilege of utilizing material in the infectious wards. I wish also to thank the internes at Cook County Hospital for their many courtesies, and also numerous physicians who aided me in obtaining material for examination.

EXPLANATION OF PLATE I.

FIG. 1.—Symbiosis—Cluster of colonies of the influenza-like bacillus around the larger staphylococcus colony. Blood-agar plate inoculated directly from pertussis sputum. Magnified 30 times.

FIG. 2.—Influenza-like bacilli from 24 hour blood-agar culture isolated from pertussis sputum. Some of the bacilli show polar staining. Magnified 1,200 diameters.

FIG. 3.—Phagocytosis of influenza-like bacilli in sputum of pertussis patient. Magnified 1,200 diameters.

FIG. 4.—Twenty-four cultures of influenza-like bacilli from pertussis sputum showing marked tendency to thread formation. Magnified 1,200 diameters.

FIG. 5.—Influenza-like bacilli in smear of sputum from case inoculated with these organisms. Magnified 1,200 diameters.

FIG. 6.—Phagocytosis of leucocytes by endothelial cells seen in peritoneal exudate of guinea-pig injected with the influenza-like bacilli. Magnified 1,200 diameters.

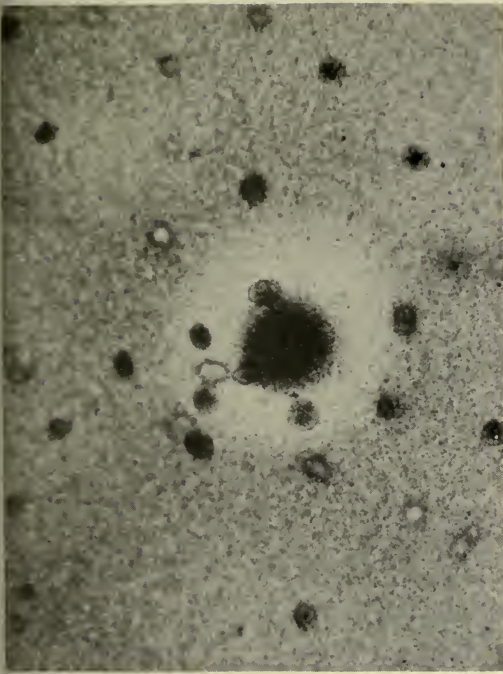


FIG. 1.



FIG. 2.

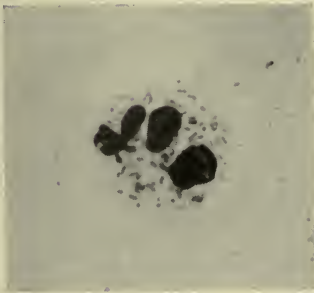


FIG. 3.

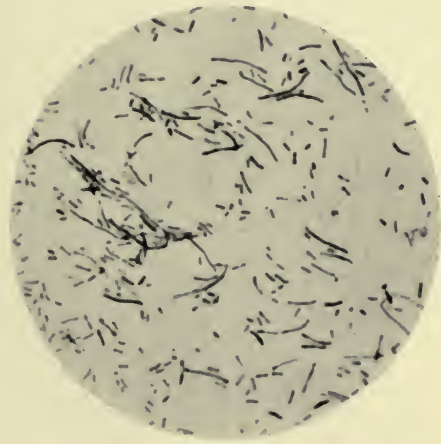


FIG. 4.

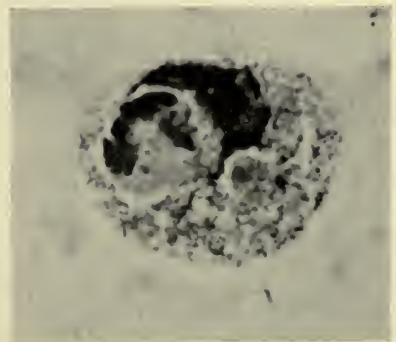
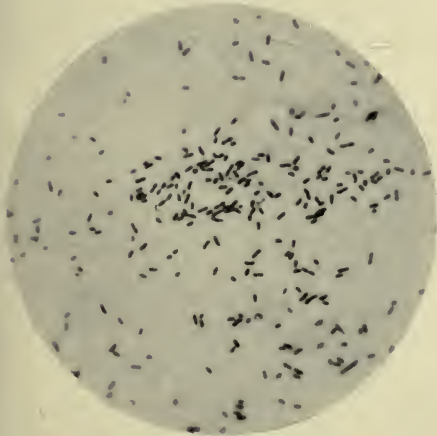


FIG. 6.



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