STUDIES ON EXPERIMENTAL PNEUMONIA.

IX. PRODUCTION IN MONKEYS OF AN ACUTE RESPIRATORY DISEASE RESEMBLING INFLUENZA BY INOCULATION WITH BACILLUS INFLUENZÆ.

By FRANCIS G. BLAKE, M.D., AND RUSSELL L. CECIL, M.D.

(From the Bacteriological Laboratories of the Army Medical School, Washington.)

(Received for publication, May 15, 1920.)

The present paper deals with the production in normal monkeys of an acute respiratory disease by inoculation of the mucous membranes of the upper respiratory tract with *Bacillus influenzae* and of bronchopneumonia by intratracheal injection.¹ These experiments were undertaken in continuation of previous studies^{2, 3, 4} on experimental pneumococcus and hemolytic streptococcus pneumonia in monkeys with the purpose of obtaining evidence concerning the relation of *Bacillus influenzae* to influenza and of determining whether the type of bronchopneumonia that has been ascribed^{5, 6, 7} to *Bacillus influenzae* infection of the lungs in man could be reproduced experimentally in animals.

In general, animal inoculation experiments with *B. influenzae*, though serving to demonstrate^{8, 9, 10} the pathogenicity of at least some strains of the organism for laboratory animals, have had little actual bearing on the relation of *B. influenzae* to influenza, since the methods of inoculation have been by the subcutaneous, intraperitoneal, or intravenous routes, and it is not to be expected that a disease

¹ Blake, F. G., and Cecil, R. L., *J. Am. Med. Assn.*, 1920, lxxiv, 170.

² Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.

³ Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 445.

⁴ Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxii, 401.

⁵ Pfeiffer, R., *Z. Hyg. u. Infektionskrankh.*, 1893, xiii, 357.

⁶ MacCallum, W. G., *J. Am. Med. Assn.*, 1919, lxxii, 720.

⁷ Wolbach, S. B., *Bull. Johns Hopkins Hosp.*, 1919, xxx, 104.

⁸ Cohen, *Ann. Inst. Pasteur*, 1909, xxiii, 273.

⁹ Wollstein, M., *Am. J. Dis. Child.*, 1911, i, 42; *J. Exp. Med.*, 1915, xxii, 445.

¹⁰ Albert, H., and Kelman, S. R., *J. Infect. Dis.*, 1919, xxv, 443.

which may safely be classified as a respiratory infection could readily be reproduced in animals by introduction of the virus by other than the natural path of infection in man. Attempts have been made, however, to produce influenza by inoculation of the mucous membranes of the respiratory tract with *B. influenza*. Pfeiffer⁵ inoculated monkeys and other laboratory animals on the mucous membranes of the upper respiratory tract, intratracheally, and by direct injection into the lung, but failed satisfactorily to reproduce influenza or to establish the fact that actual infection took place, though many of the animals suffered from a temporary intoxication and sometimes died. Wollstein and Meltzer¹¹ produced bronchopneumonic lesions in dogs by massive intrabronchial insufflation of *B. influenza*. Wahl, White, and Lyall¹² inoculated the nasal and nasopharyngeal mucous membranes of healthy men with four strains of *B. influenza* with negative results. Similar experiments conducted by the United States Public Health Service¹³ likewise yielded negative results.

Strain of Bacillus influenza Used.

Since it seemed apparent that there was no adequate method available for distinguishing between possible saprophytic and pathogenic varieties of Gram-negative hemophilic bacilli to which the name *Bacillus influenza* is at present indiscriminately applied, it seemed desirable to employ a single strain which could be considered with reasonable certainty to be the infecting organism in the case from which it was isolated. Accordingly, the strain of *Bacillus influenza*¹⁴ used, designated B, was one originally isolated in pure culture from the pleural exudate withdrawn from a case of empyema following influenzal pneumonia in a child. It conformed to such criteria as we possess for identifying *Bacillus influenza* in that it was a small, Gram-negative, non-motile, non-spore-bearing, non-hemolytic bacillus, growing readily in characteristic colonies on suitable hemoglobin-containing media at 37°C., but failing to grow in the absence of hemoglobin or at 20°C. It produced indole. When received it had been grown on artificial media for about 6 weeks and was found to possess practically no virulence for white mice, the 18 hour growth on a whole brown blood agar plate not being lethal on intraperitoneal inoculation.

¹¹ Wollstein, M., and Meltzer, S. J., *J. Exp. Med.*, 1912, xvi, 126.

¹² Wahl, H. R., White, G. B., and Lyall, H. W., *J. Infect. Dis.*, 1919, xxv, 419.

¹³ *Public Health Rep., U. S. P. H.*, 1919, xxxiv, 33.

¹⁴ We are indebted to Dr. T. M. Rivers of the Johns Hopkins Medical School, Baltimore, for the strain of *B. influenza* used.

Method of Increasing the Virulence of Bacillus influenzae.

A preliminary intratracheal test inoculation of a monkey having resulted in no evidence of infection, it was evident that the virulence of the strain of *Bacillus influenzae* would have to be raised if successful results were to be obtained. This was accomplished by successive animal passages through a series of eleven white mice followed by a series of thirteen monkeys. These animals were inoculated intraperitoneally with broth or 0.85 per cent salt solution suspensions of *Bacillus influenzae* made from brown blood agar plate cultures. At the beginning of the mouse series the growth from one-half of a plate culture was inoculated. 6 to 8 hours after inoculation the mouse was killed and a plate culture of the peritoneal exudate was made for inoculation of the next mouse on the following day. This procedure was adopted because a few preliminary experiments had shown that the influenza bacillus began to disappear from the peritoneal exudate by 8 hours after inoculation and was entirely gone by 24 hours. With the successive inoculations the organism became progressively more virulent for mice until at the end of the series the growth on one-fourth of a plate killed a mouse in 6 to 8 hours with peritonitis and septicemia.

Following the mouse series the organism was passed by similar methods through thirteen monkeys. The monkeys received large inoculations varying from the growth on four to that on fourteen plates. Cultures for the next succeeding inoculation were made either from peritoneal exudate withdrawn by a sterile capillary pipette 6 to 8 hours after intraperitoneal injection, or from the peritoneal exudate at autopsy in the monkeys that succumbed. Some delay occurred in this series owing to secondary invasion of the peritoneal cavity by *Staphylococcus albus* in some of the monkeys. When this occurred it was necessary to prepare subcultures for the next passage in order to eliminate the staphylococcus. The symptoms produced by the inoculations were those of peritonitis and profound intoxication. In monkeys in which leucocyte counts were made a rapidly developing and very marked leucopenia was found, the counts falling in 3 to 6 hours after inoculation from the normal range of 15,000 to 25,000 down to 2,500 to 10,000 per c. mm., and remaining low until death or recovery took place. The series of passages is shown in Table I.

TABLE I.
Passage of *B. influenza* through Monkeys by Intraperitoneal Inoculation.

Passage monkey No.	Date of inoculation.	Culture of <i>B. influenza</i> .	Amount inoculated.	Result.	Culture of peritoneal exudate.	
					6-8 hrs. after inoculation.	Autopsy.
P 1	1919 .					
" 2	July 29	B. 11 ^{1*}	7 plates.	Recovered.	<i>B. influenza</i> .	
" 3	" 30	" 11.1 ¹	4 "	"	"	
" 4	Aug. 2	" 11.2 ³	9 "	Aug. 3. Died.	<i>Staphylococcus albus</i> .	
" 5	" 3	" 11.3 ¹	5½ "	" 4. "	<i>B. influenza</i> .	<i>B. influenza</i> .
" 6	" 6	" 11.4 ²	8 "	" 7. "	"	"
" 7	" 7	" 11.5 ¹	5 "	Recovered.	"	<i>Staphylococcus albus</i> .
" 8	" 9	" 11.6 ²	12 "	Aug. 10. Died.	"	<i>B. influenza</i> .
" 9	" 12	" 11.7 ²	11 "	" 14. "	"	"
" 10	" 14	" 11.8 ²	12 "	" 15. Killed.	"	"
" 11	" 16	" 11.9 ²	14 "	Recovered.	"	"
" 12	" 19	" 11.10 ²	10 "	"	<i>Staphylococcus albus</i> .	
" 13a	" 20	" 11.11 ¹	10 "	Aug. 20. Killed.	<i>B. influenza</i> .	<i>B. influenza</i> .
" 13b	" 22	" 11.12 ²	10 "	Recovered.	<i>B. influenza</i> .	
	" 25	" 11.12 ²	12 "	Aug. 26. Died.	Diphtheroid bacillus.	<i>B. influenza</i> .

* B. 11 indicates *B. influenza*, Strain B, 11 mouse passages. B. 11.1, etc., indicate Strain B, 11 mouse passages, 1 monkey passage, etc. The exponents indicate the number of cultures removed from the last passage animal.

At the completion of the series 0.1 cc. of a 16 hour blood broth culture of *Bacillus influenzae* from the peritoneal exudate of Passage Monkey 13b killed a white mouse within 48 hours, 1 cc. in 5 hours. To indicate the rapidity with which this increased virulence was lost by subculture on artificial media, it may be stated that after five transplants in blood broth at 24 hour intervals 1 cc. failed to kill a mouse.

Method.

Two species of monkeys were used, *Cebus capucinus* and *Macacus syrichtus*. All were normal healthy animals as far as could be determined by preliminary observation. No procedures to lower resistance or to injure the respiratory tract were resorted to before inoculation. It is well recognized¹⁵ that the use of monkeys for experimental purposes requires great care in the interpretation of febrile reactions and of variations in the leucocyte count as evidences of infection. To establish the production of disease in the monkey the summation of clinical symptoms, temperature, and leucocyte reactions and the results of bacteriological and pathological examinations are essential. This is particularly true in the case of inoculations with *Bacillus influenzae*, since it is clearly necessary, in view of the fairly well established toxic effects of influenza bacillus cultures and culture filtrates,^{16,17} to show that the results obtained are due to actual infection with *Bacillus influenzae* and are not merely the result of a temporary intoxication. The methods of study after inoculation, therefore, were the careful observation and recording of clinical symptoms, the taking of morning and evening rectal temperatures, daily total and differential counts of the leucocytes, microscopic examination of stained films of the respiratory tract secretions, and the taking of cultures of the nasal secretion and blood at irregular intervals. Since the disease produced did not prove fatal, most of the animals were killed by a sharp blow on the skull either during the acute stage of the disease or shortly after apparent recovery, and autopsies with bacteriological examination were immediately performed. Whenever

¹⁵ Draper, G., and Hanford, J. M., *J. Exp. Med.*, 1913, xvii, 517.

¹⁶ Parker, J. T., *J. Immunol.*, 1919, iv, 331.

¹⁷ Huntoon, F. M., and Hannum, S., *J. Immunol.*, 1919, iv, 167.

Bacillus influenzae was recovered in cultures during life or at autopsy it was identified as such by appropriate cultural tests.

Because of the extraordinary rapidity with which *Bacillus influenzae* may lose its virulence when subcultured on artificial media outside the animal body, a particular effort was made to inoculate the monkeys with cultures as recently removed as possible from the passage monkeys. The material used for inoculation consisted of (1) first or second subculture on brown blood agar plates or in blood broth of *Bacillus influenzae* recovered from the peritoneal exudate of monkeys of the passage series described above; (2) original or first subculture of *Bacillus influenzae* recovered from the respiratory tract of a monkey (No. 138) killed during the acute stage of the pneumonia produced by intratracheal injection of the organism; or (3) the peritoneal exudate of monkeys of the passage series dying of *Bacillus influenzae* peritonitis and septicemia. Comparatively young cultures—10 to 16 hours incubation—were used for inoculation. When plate cultures were employed the bacteria were washed off and suspended in sterile broth so that 1 cc. was equivalent to the growth on one plate. All cultures used for inoculation were tested for purity and identified as *Bacillus influenzae*. In using peritoneal exudate directly the absence of bacterial contamination was controlled by microscopic examination of stained films before inoculation, and the material was subsequently shown by culture to contain only *Bacillus influenzae*. The exudate was inoculated at varying intervals within an hour after removal at autopsy, being kept meanwhile at 37°C.

Two methods of inoculation were employed with different purposes in view. In one series of monkeys in order to determine whether *Bacillus influenzae* would initiate an infection of the upper respiratory tract and, if so, what the characteristics of the disease produced might be, the material to be inoculated was introduced into the nose and mouth either by application to the mucous membranes with a cotton swab previously dipped in the culture material or by instillation with a pipette. In a second series of monkeys the material was introduced into the lower respiratory tract by direct intratracheal injection according to the method previously described.² The purpose of this series of experiments was to determine whether pneumonia could be experimentally produced in monkeys with *Bacillus influenzae*, and, if

so, whether it would present the pathologic characteristics that have been ascribed to pure *Bacillus influenzae* pneumonia in man by Pfeiffer,⁵ MacCallum,⁶ Wolbach,⁷ and others.

EXPERIMENTAL.

Effect of Inoculating Monkeys on the Mucous Membranes of the Upper Respiratory Tract with Bacillus influenzae.

Twelve monkeys received inoculations of *Bacillus influenzae* on the mucous membranes of the nose or nose and mouth (Table II). The amount of culture or peritoneal exudate used in this series of experiments varied from what could be introduced by a single application in each nostril and the mouth of a cotton swab previously dipped in the material to be inoculated, up to 1 cc. of a blood broth culture of *Bacillus influenzae* instilled into each nostril and 1 cc. into the mouth by a pipette. In every instance there resulted an acute respiratory infection which appeared to be essentially similar to influenza in man.

Clinical Course and Symptoms.—The onset occurred quite suddenly from 3 to 5 hours after inoculation. The first symptom was a variable degree of prostration, often extreme, the animals in many cases lying flat on the floor of the cage, eyes closed and very stuporous, sometimes pressing their hands tightly on the top of the head. This picture was in striking contrast with the onset of pneumococcus or streptococcus pneumonia in monkeys, for in these diseases prostration at onset was never observed, and it was frequently difficult to recognize by inspection that a monkey had become sick except for the accelerated respiration. Symptoms of upper respiratory tract infection soon followed. Frequent sneezing, blinking of the eyes, and rubbing of the nose were the prominent manifestations.

The subsequent course of the disease, though showing some variation in different monkeys, particularly with respect to the development of complications, was in general that of a self-limited infection, the acute stage of which lasted from 3 to 5 days. By the end of 24 hours a variable amount of nasal secretion had appeared, at first mucoid and scanty, later in some instances becoming more profuse and mucopurulent. Stained films and cultures of this secretion in some cases failed to show *Bacillus influenzae* or at most showed only small num-

TABLE II.
Effect of Inoculating Monkeys on the Mucous Membranes of the Upper Respiratory Tract with *B. influenza*.

Monkey No.	Species.	Weight. gm.	Date of inoculation. 1919	Material inoculated.	Strain of <i>B. influenza</i> inoculated.	Amount inoculated.		Result.	Complications.	<i>B. influenza</i> recovered at autopsy.			
						Nose. cc.	Mouth. cc.			U. R. T.	L. R. T.	T.	
150	<i>C. capucinus</i> .	1,350	Aug. 25	10 hr. blood broth culture.	B. 11.11 ² *	2.0	1.0	Acute respiratory disease. Improving on 4th day; killed.		-	+		
154	"	1,740	" 26	Peritoneal exudate of Monkey P 13b.	" 11.13 ^o	Swab.	Swab.	Acute respiratory disease. Improving on 4th day; killed.			+		
153	"	950	" 26	" "	" 11.13 ^o	1.0		Acute respiratory disease. Killed on 4th day.	Acute sinusitis, left antrum.	-			
140	"	1,600	" 26	" "	" 11.13 ^o	Swab.	Swab.	Acute respiratory disease. Improving on 4th day; killed.		-	+		
148	"	2,160	" 26	" "	" 11.13 ^o	"	"	Acute respiratory disease. Killed on 3rd day.		+	-		
142	"	1,300	" 26	" "	" 11.13 ^o	0.5		Acute respiratory disease. Killed on 4th day.	Acute sinusitis, left antrum; bronchopneumonia, R. L.	-			
139	"	1,405	" 26	" "	" 11.13 ^o	0.5		Acute respiratory disease. Killed on 3rd day.	Acute sinusitis, both antra.	+			

157	<i>M. syrichius</i>	5,200	Aug. 26	Peritoneal exudate of Mon- key P 13b.	B. 11.13°	2.0	1.0	Acute respiratory dis- ease. Killed on 4th day.	Acute sinusitis, both antra. Bronchio- litis; bronchopneu- monia, L. U., R. U., R. L.	+	+
158	"	5,675	"	27 15 hr. blood broth cul- ture.	" 11.13°	2.0	1.0	Acute respiratory dis- ease. Recovered by 6th day.	No autopsy.		
159	"	5,430	"	27 "	" 11.13°	2.0	1.0	Acute respiratory dis- ease. Killed on 6th day.	Acute sinusitis, both antra; bronchioli- tis; bronchopneu- monia, R. U., L. U., L. M., L. L.	+	+
160	<i>C. capucinus</i>	1,500	"	27 "	" 11.13°	1.5	1.0	Acute respiratory dis- ease. Recovered by 6th day.	No autopsy.		
161	"	1,340	"	27 "	" 11.13°	1.5	1.0	Acute respiratory dis- ease. Recovered by 6th day.	"	"	"

* This culture was a strain of *B. influenzae* recovered at autopsy from Monkey 138 (Table III).

R. L., R. U., etc., indicate lobes of the lungs. U. R. T. indicates upper respiratory tract, including the antra; L. R. T., lower respiratory tract.

bers phagocytosed by polymorphonuclear leucocytes; in other cases *Bacillus influenzae* was present in immense numbers. By 24 to 48 hours after inoculation the infection had spread to the lower respiratory tract, as evidenced by the development of a racking cough which suggested an inflammatory irritation of the trachea and bronchi. This symptom was striking in comparison with the cough of monkeys with pneumococcus pneumonia in which coughing occurred only late in the disease and was comparatively infrequent. That it was due to actual infection of the trachea by *Bacillus influenzae* was shown by the recovery of the influenza bacillus from the trachea at autopsy, either in pure culture or in association with bacteria that are normally present in the mouths of monkeys.

The febrile reaction generally was not great. Four animals showed no elevation throughout the course of the disease. The character of the temperature curve was inconstant and bore no apparent relation to the severity of the infection, the afebrile animals being as sick as those that showed elevation of temperature. One fairly constant feature was a fall in temperature on the 2nd or 3rd day followed by a secondary rise which appeared to be associated with the development of tracheobronchitis.

The leucocyte counts during the active stage of the disease showed either a definite degree of leucopenia or no significant variation from the normal. The differential counts showed a very brief preliminary polymorphonuclear percentage increase followed by a fall to normal or subnormal level. In two cases a leucocytosis developed on the 2nd and 4th days respectively, autopsies in these instances showing a complicating purulent sinusitis.

Complications.—An acute purulent sinusitis affecting one or both antra developed in five monkeys. At autopsy the antra were found to contain a purulent exudate and the mucous membranes were edematous and congested. Cultures from the antra showed abundant influenza bacilli in three cases, twice in pure culture. In the other two none was recovered, although in one small numbers were seen in stained films of the exudate. In three instances other bacteria which are normal inhabitants of the upper respiratory tract of monkeys were also present.

Two monkeys (Nos. 157 and 159) developed a clinically recognizable bronchopneumonia on the 3rd and 4th days respectively. The onset of pneumonia was insidious and was suspected only because of a rise in temperature and a moderate acceleration of the respiratory rate. Both animals were killed early in the course of the pneumonia and *Bacillus influenzae* was recovered in pure culture from the lungs. A third monkey (No. 142) developed a small patch of bronchopneumonia which was not recognized during life. The pathology of the disease and its complications are described in the following paper.¹⁸

Experiment 1.—Monkey 150 (Text-fig. 1, a). *Cebus capucinus*, male; weight 1,350 gm. Aug. 23 and 24, 1919. Well and active. Aug. 25, 10.30 a.m. 1 cc. of 10 hour blood broth culture of *B. influenzae* (Strain B. 11.11²) instilled into each nostril and 1 cc. into mouth with pipette. 2 p.m. Appears very sick and stuporous, lying curled up in corner of cage, paying little attention to surroundings; eyes dull; blinks lids frequently; presses hands on top of head; sneezes occasionally. 4.30 p.m. Condition the same; blood culture, no growth. 8 p.m. Sneezes very frequently. Aug. 26, 11 a.m. Still sick but less prostrated; refuses food; sneezes frequently; moderate mucoid nasal discharge; throat injected; stained film of nasal secretion shows many *B. influenzae*, mostly phagocytosed; throat culture, *B. influenzae*, non-hemolytic streptococci. 8 p.m. Condition the same. Aug. 27. Still sick, but appears better; sneezes occasionally; coughs occasionally; respirations normal. Aug. 28. Improving; sneezes and coughs occasionally. 10 a.m. Killed.

Autopsy. Anatomical Diagnosis.—Acute rhinitis; acute tracheobronchitis; hyperplasia of thymus.

Cultures.—Heart's blood, no growth; nasal mucous membranes, *Staphylococcus albus*; trachea, *B. influenzae* and *Streptococcus viridans*.

Experiment 2.—Monkey 154 (Text-fig. 1, b). *Cebus capucinus*, male; weight 1,740 gm. Aug. 25, 1919. Well and active. Aug. 26, 11.15 a.m. Mucous membranes of nose and throat swabbed with cotton applicator previously dipped in salt solution washings from peritoneal cavity of Passage Monkey 13b (Strain B. 11.13⁰). 1.30 p.m. Quiet; head drooping; eyes closed; sneezes occasionally. 4 p.m. Refuses food. 8 p.m. Sneezes very frequently. Aug. 27, 10 a.m. Quiet; sneezes very frequently. Moderate mucopurulent nasal discharge; stained film shows many polymorphonuclear leucocytes, few *B. influenzae*, and Gram-positive cocci. 8 p.m. Condition the same; shakes head and rubs nose frequently. Aug. 28. Appears better; sneezes frequently and coughs occasionally. Aug. 29. Appears weak and exhausted; coughs frequently. Killed.

Autopsy. Anatomical Diagnosis.—Acute rhinitis; acute tracheobronchitis; hyperplasia of thymus.

¹⁸ Cecil, R. L., and Blake, F. G., *J. Exp. Med.*, 1920, xxxii, 719.

Cultures.—Heart's blood, no growth; trachea, *B. influenzae*, *Streptococcus viridans*, and Gram-negative micrococcus.

Experiment 3.—Monkey 153 (Text-fig. 1, c). *Cebus capucinus*, female; weight 950 gm. Aug. 25, 1919. Well and active. Aug. 26, 11 a.m. 0.5 cc. of salt solution washings from peritoneal cavity of Passage Monkey 13b (Strain B. 11.13⁰)

TEXT-FIG. 1, a to c. Acute respiratory disease produced by inoculation of the mucous membranes of the upper respiratory tract with *B. influenzae*. (a) Monkey 150. (b) Monkey 154. (c) Monkey 153; complicated by sinusitis.

instilled into each nostril with pipette. 1.30 p.m. Appears stuporous; head drooping. 4 p.m. Very sick; lying prostrate on floor of cage; refuses food. 8 p.m. Sneezes occasionally. Aug. 27. Still sick but appears better; sneezes frequently. Moderate mucoid nasal discharge; stained film shows moderate number of polymorphonuclear leucocytes, lymphocytes, and epithelial cells but

no bacteria. Aug. 28. Quiet; head drooping; eyes closed much of time; sneezes frequently. Aug. 29. Still sick; no animation; sneezes and coughs occasionally. Killed.

Autopsy. Anatomical Diagnosis.—Acute rhinitis; acute purulent sinusitis, left antrum; acute tracheobronchitis; hyperplasia of thymus.

Stained Films.—Tracheal exudate, many leucocytes and epithelial cells, few *B. influenzae*; left antrum, abundant leucocytes, few *B. influenzae*.

Cultures.—Heart's blood, trachea, and left antrum, no growth.

In the following experiment the effect of exposure to cold and wet on the subsequent course of the disease was studied. Monkeys 148 and 139 were exposed to cold and wet for 4 hours after inoculation by sprinkling them with cold water from time to time and by directing a current of air on them with an electric fan. Monkeys 140 and 142, not subjected to exposure, served as controls.

Experiment 4.—Monkey 140 (Text-fig. 2, a). *Cebus capucinus*, female; weight 1,600 gm. Aug. 23 to 25, 1919. Well and active. Aug. 26, 10.10 a.m. Mucous membranes of nose and throat swabbed with cotton applicator previously dipped in peritoneal exudate of Passage Monkey 13b (Strain B. 11.13⁰). 1.30 p.m. Very quiet. 4 p.m. Quiet; head drooping; sneezes occasionally. Aug. 27. Moderately sick; refuses food; sneezes occasionally. Scanty mucoid nasal discharge; stained film shows leucocytes and epithelial cells but no bacteria; culture, no *B. influenzae*. Aug. 28. Appears sicker; lying down; very drowsy; coughs and sneezes frequently. Aug. 29. Appears better but is quiet and exhausted; sneezes occasionally; coughs frequently. Killed.

Autopsy. Anatomical Diagnosis.—Acute rhinitis; acute tracheobronchitis.

Cultures.—Heart's blood, no growth; nasal mucous membranes, *Staphylococcus albus* and large Gram-negative bacillus; trachea, *B. influenzae*, *Staphylococcus albus*, and *Streptococcus viridans*.

Monkey 148 (Text-fig. 2, b). *Cebus capucinus*, female; weight 2,160 gm. Aug. 23 to 25, 1919. Well and active. Aug. 26, 10.20 a.m. Mucous membranes of nose and throat swabbed with cotton applicator previously dipped in peritoneal exudate of Passage Monkey 13b (Strain B. 11.13⁰). 11 a.m. to 3 p.m. Exposure to cold and wet. 1.30 p.m. Very sick and stuporous, head drooping; eyes closed; shivering. 4 p.m. Prostrated; offers no resistance on handling; refuses food. Aug. 27. Very sick and prostrated; sneezes occasionally. Mucopurulent nasal secretion shows many leucocytes and epithelial cells and a few phagocytosed *B. influenzae*. Aug. 28. Very sick; lying prostrate; sneezes frequently; no cough. Killed.

Autopsy. Anatomical Diagnosis.—Acute rhinitis; hyperplasia of thymus.

Cultures.—Heart's blood, trachea, and lungs, no growth; nasal mucous membranes, abundant *B. influenzae* and few *Staphylococcus albus*.

TEXT-FIG. 2, *a* to *d*. Acute respiratory disease produced by inoculation of the mucous membranes of the upper respiratory tract with *B. influenzae*. (*a*) Monkey 140. (*b*) Monkey 148. (*c*) Monkey 142; complicated by sinusitis and bronchopneumonia. (*d*) Monkey 139; complicated by sinusitis.

Monkey 142 (Text-fig. 2, *c*). *Cebus capucinus*, female; weight 1,300 gm. Aug. 23 to 25, 1919. Well and active. Aug. 26, 10.30 a.m. 0.25 cc. of peritoneal exudate from Passage Monkey 13b (Strain B. 11.13⁰) instilled into each nostril with pipette. 1.30 p.m. Quiet; curled up in corner of cage; head drooping; eyes closed. 4 p.m. Quite sick and stuporous; refuses food; sneezes frequently. Aug. 27. Moderately sick; sneezes frequently. Mucopurulent nasal discharge shows many leucocytes and epithelial cells; no bacteria in stained film. Aug. 28. Still sick; sneezes very frequently. Aug. 29. Appears weak and without animation; sneezes occasionally. Killed.

Autopsy. Anatomical Diagnosis.—Acute rhinitis; acute sinusitis, left antrum; bronchopneumonia, right lower lobe.

Cultures.—Heart's blood and trachea, no growth; left antrum, few *Streptococcus viridans*.

Monkey 139 (Text-fig. 2, *d*). *Cebus capucinus*, female; weight 1,405 gm. Aug. 23 to 25, 1919. Well and active. Aug. 26, 10.40 a.m. 0.25 cc. of peritoneal exudate from Passage Monkey 13b (Strain B. 11.13⁰) instilled into each nostril with pipette. 11 a.m. to 3 p.m. Exposure to cold and wet. 4 p.m. Very sick; head drooping; stuporous; eyes closed; shivering; refuses food. Aug. 27. Very sick; lying down; sneezes occasionally. Profuse mucopurulent nasal discharge showing abundant leucocytes, many *B. influenzae*, and few staphylococci; culture, *B. influenzae* and *Staphylococcus albus*. Aug. 28. Quite sick and stuporous; sneezes frequently and rubs nose; mucopurulent nasal discharge continues. 1.30 p.m. Killed.

Autopsy. Anatomical Diagnosis.—Acute rhinitis; acute purulent sinusitis, both antra; acute tracheobronchitis; hyperplasia of thymus.

Cultures.—Heart's blood and trachea, no growth; both antra, abundant *B. influenzae*.

Experiment 5.—Monkey 157 (Text-fig. 3, *a*). *Macacus syrichtus*, male; weight 5,200 gm. Aug. 25, 1919. Well, active, and ferocious. Aug. 26, 11.25 a.m. 1 cc. of salt solution washings of peritoneal cavity of Passage Monkey 13b (Strain B. 11.13⁰) instilled into each nostril and 1 cc. into mouth. 2.30 p.m. Quite sick; lying down; stuporous; eyes closed. 4 p.m. Very sick; head drooping; refuses food. 10 p.m. Vomited. Aug. 27. Sick, no fight. Moderate mucopurulent nasal discharge; stained films show many polymorphonuclear leucocytes, many of them containing from two to twelve phagocytosed *B. influenzae*; culture, no *B. influenzae*. 2 p.m. Coughing violently; respirations not accelerated. Aug. 28. Still sick; sitting huddled up in corner of cage paying no attention to surroundings; coughs frequently; respirations moderately accelerated. Aug. 29. No animation; respirations accelerated; frequent cough. 11 a.m. Killed.

Autopsy. Anatomical Diagnosis.—Acute rhinitis; acute bilateral sinusitis of antra; acute tracheobronchitis; hemorrhagic edema of lungs; bronchiolitis; bronchiectasis; bronchopneumonia, left upper and right upper and lower lobes; hyperplasia of thymus.

Cultures.—Heart's blood and trachea, no growth; nasal mucous membrane, *B. influenza* and *Staphylococcus albus*; right antrum, *B. influenza* and Gram-negative micrococcus; right and left bronchi and right lower and upper lobes, pure culture of *B. influenza*; left upper lobe, *Streptococcus viridans*.

TEXT-FIG. 3, a to c. Acute respiratory disease produced by inoculation of the mucous membranes of the upper respiratory tract with *B. influenza*. (a) Monkey 157; complicated by sinusitis and bronchopneumonia. (b) Monkey 158. (c) Monkey 159; complicated by sinusitis and bronchopneumonia.

Experiment 6.—Monkey 158 (Text-fig. 3, b). *Macacus syrichtus*, male; weight 5,675 gm. Aug. 26, 1919. Well and ferocious. Aug. 27, 11 a.m. 3 cc. of 15 hour blood broth culture of *B. influenza* (Strain B. 11.13³) instilled into nose and mouth with pipette. 4 p.m. Quiet. Aug. 28. Appears moderately sick but without definite symptoms. Aug. 29 and 30. Condition the same; has developed a tight racking cough. Aug. 31. More active; coughs. Sept. 1. Appears well and active, but coughs occasionally. Observation discontinued.

Experiment 7.—Monkey 159 (Text-fig. 3, c). *Macacus syrichtus*, male; weight 5,430 gm. Aug. 26, 1919. Well, active, and ferocious. Aug. 27, 11.15 a.m. 3 cc. of 15 hour blood broth culture of *B. influenza* (Strain B. 11.13³) instilled into nose and mouth with pipette. 2 p.m. Appears sick; resting against side of cage. Aug. 28. Quiet. Muroid nasal discharge; stained films show many leucocytes, some containing two to four *B. influenza*. Aug. 29. Has developed a dry racking cough; nasal discharge continues. 5 p.m. Appears quite sick; no animation; leaning against side of cage. Aug. 30 and 31. Condition the same; rubs eyes and nose and sneezes occasionally; coughs frequently; respirations accelerated. Sept. 1. Sick and exhausted; breathing accelerated; sneezes and coughs. 10.30 a.m. Killed.

Autopsy. Anatomical Diagnosis.—Acute rhinitis; acute bilateral sinusitis of antra; acute tracheobronchitis; hemorrhagic edema of lungs; bronchiolitis; bronchopneumonia, right upper and left upper, middle, and lower lobes; hyperplasia of thymus.

Cultures.—Heart's blood and left middle lobe, no growth; right antrum, pure culture of *B. influenza*; left antrum, *B. influenza* and *Streptococcus viridans*; trachea, *B. influenza*, *Streptococcus viridans*, and Gram-negative micrococcus; right and left bronchi, *B. influenza* and Gram-negative micrococcus; left lower and upper lobes, pure culture of *B. influenza*.

Experiment 8.—Monkey 160 (Text-fig. 4, a). *Cebus capucinus*, male; weight 1,500 gm. Aug. 26, 1919. Well and active. Aug. 27, 11.25 a.m. 2.5 cc. of 15 hour blood broth culture of *B. influenza* (Strain B. 11.13³) instilled into nose and mouth with pipette. 3 p.m. Sick; lying flat; rubs eyes; sneezes frequently. Aug. 28. Sick but less prostrated; sneezes and rubs eyes and nose frequently. Muroid nasal discharge; stained film shows leucocytes and epithelial cells but no bacteria. Aug. 29. Condition the same. 3 p.m. Has begun to cough. Aug. 31. Appears better; sneezes and coughs occasionally. Sept. 1. Appears well. Observation discontinued.

Experiment 9.—Monkey 161 (Text-fig. 4, b). *Cebus capucinus*, male; weight 1,340 gm. Aug. 26, 1919. Well and active. Aug. 27, 11.35 a.m. 2.5 cc. of 15 hour blood broth culture of *B. influenza* (Strain B. 11.13³) instilled into nose and mouth with pipette. 1 p.m. Begins to appear sick. 2 p.m. Very sick; lying prostrate. 4 p.m. Lying down; stuporous; eyes closed; sneezes frequently. Aug. 28. Sick though less prostrated; mucopurulent nasal discharge; sneezes frequently. Aug. 29 and 30. Condition the same; coughs occasionally. Aug. 31. Appears better; coughs and sneezes occasionally. Sept. 1. Improving; observation discontinued.

In order to determine whether the culture medium in which *Bacillus influenza* had grown would exert any toxic effect when inoculated on the mucous membranes of the upper respiratory tract the following experiment was carried out.

Experiment 10.—Monkey 156. *Cebus capucinus*, female; weight 1,120 gm. Aug. 26, 1919. Well and active. 3 cc. of supernatant broth of a 16 hour blood broth culture of *B. influenza* (Strain B. 11.13²) freed from bacteria by centrifugalization instilled into nose and mouth with pipette. Monkey remained well and active, exhibiting no symptoms throughout a period of 6 days when observation was ended.

TEXT-FIG. 4, *a* and *b*. Acute respiratory disease produced by inoculation of the mucous membranes of the upper respiratory tract with *B. influenza*. (*a*) Monkey 160. (*b*) Monkey 161.

Effect of Intratracheal Injection of Bacillus influenza.

Ten monkeys were injected intratracheally with *Bacillus influenza* of enhanced virulence (Table III). These experiments may properly be divided into two groups of five animals each. In the first group

(Monkeys 124, 20, 137, 138, and 149) massive doses of *Bacillus influenzae* were employed in an attempt further to increase the virulence of the organism for the respiratory tract of monkeys if possible. This did not prove feasible, however, and was abandoned. Three of these animals developed tracheobronchitis and bronchopneumonia, one bronchitis and bronchopneumonia, and one tracheobronchitis without pneumonia. In one instance (Monkey 138) *Bacillus influenzae* pericarditis and septicemia ensued. Although all these animals were unquestionably infected with *Bacillus influenzae* the value of the results is lessened because of the large doses employed. This is shown by the fact that a control monkey (No. 155) injected intratracheally with an equivalent amount of *Bacillus influenzae* previously killed by heating at 56°C. for 1 hour, although showing none of the other evidences of actual infection exhibited by the monkeys inoculated with living cultures, did show moderate bronchopneumonic lesions at autopsy. In the second group (Monkeys 108, 136, 135, 141, and 152) a comparatively small dose (1 cc.) was employed with the successful production of bronchopneumonia in three animals. One developed tracheobronchitis without pneumonia and one resisted infection. In this group the development of pneumonia was clearly dependent upon actual infection with *Bacillus influenzae*.

The course and general symptoms of the disease produced in these animals were similar to those occurring in the monkeys inoculated in the upper respiratory tract; that is, a self-limited respiratory disease of 3 to 6 days duration, sudden onset after a brief incubation period, prostration, a variable febrile reaction, and leucopenia, or no significant change in the leucocyte count. Leucocytosis with pericarditis occurred in Monkey 138. The disease was accompanied by a frequent and severe racking cough and accelerated respiratory rate. In no case did the disease prove fatal. It seems probable, however, that death would have occurred in the monkey that developed pericarditis and septicemia had it not been killed.

The nine monkeys showing clinical evidence of infection were killed at varying intervals after inoculation for pathological and bacteriological examination. In three of the seven monkeys which developed pneumonia *Bacillus influenzae* was recovered in pure culture from the lungs or bronchi, in four the cultures remained sterile, indicating that

TABLE III.
Effect of Intratracheal Injection of B. influenzae.

Monkey No.	Species.	Weight. gm.	Date of injection.	Material injected.	Strain of <i>B. influenzae</i> injected.	Amount injected. cc.	Result.	Autopsy cultures.
124	<i>M. syrichtus</i> .	1,500	1919 Aug. 11	Broth suspension of 16 hr. plate culture.	B. 11.7 ²	5.0	Tracheobronchitis; bronchopneumonia. Killed on 3rd day.	H. B., n. g. Tr., B. I. Lungs, B. I.
20	"	3,500	" 14	Broth suspension of 12 hr. plate culture.	" 11.8 ²	5.0	Tracheobronchitis. Recovered by 7th day. Killed.	H. B., n. g. Tr., B. I.
137	"	2,830	" 19	"	" 11.10 ²	4.0	Tracheobronchitis; bronchopneumonia. Killed on 3rd day.	H. B., n. g. Tr., " Lungs, "
138	"	2,915	" 19	"	" 11.10 ²	4.0	Tracheobronchitis; bronchopneumonia; acute pericarditis; septicemia. Killed on 3rd day.	H. B., B. I. Tr., " Lungs, " Pericard., " H. B., n. g.
149	<i>C. capucinus</i> .	1,500	" 23	"	" 11.11 ^{2*}	4.0	Bronchiolitis; bronchopneumonia. Recovered by 6th day. Killed.	Tr., " Lungs, " H. B., n. g.
108	<i>M. syrichtus</i> .	2,230	" 14	"	" 11.8 ²	1.0	Tracheobronchitis. Recovered by 6th day. Killed.	Tr., " H. B., "
136	<i>C. capucinus</i> .	1,100	" 14	Peritoneal exudate of Monkey P 8.	" 11.8 ⁰	1.0	Tracheobronchitis; hemorrhagic edema of lungs; bronchopneumonia. Killed on 6th day.	Br., B. I. Lungs, n. g.
135	<i>M. syrichtus</i> .	2,150	" 14	Peritoneal exudate of Monkey P 9.	" 11.9 ⁰	1.0	No disease.	

141	<i>C. capucinus</i> .	1,400	Aug. 22	Broth suspension of 15 hr. plate culture.	B. 11.11 [*]	1.0	Tracheobronchitis; bronchopneumonia; convalescent by 8th day. Killed.	H. B., n. g. Tr., " Turb., Staph. A.
152	"	1,035	" 26	Peritoneal exudate of Monkey P 13b.	" 11.13 ^o	1.0	Tracheobronchitis; bronchopneumonia. Killed on 2nd day.	H. B., n. g. Tr., " Lungs, "

* This culture was a strain of *B. influenza* recovered from Monkey 138 at autopsy.

H. B. indicates heart's blood; Tr., trachea; Br., bronchi; turb., turbinate; B. I., *B. influenza*; n. g., no growth; Staph. A., *Staphylococcus albus*.

the infection had terminated. In the two animals in which only a tracheobronchitis developed, cultures from the trachea at autopsy yielded *Bacillus influenzae* in pure culture in one, no growth in the other. Other bacteria, normal inhabitants of the upper respiratory tract of monkeys, were not found in this series. Their total absence

TEXT-FIG. 5. Monkey 20. Tracheobronchitis following intratracheal injection of *B. influenzae*.

was in striking contrast with their frequent presence in the trachea in the series of monkeys inoculated on the mucous membranes of the upper respiratory tract in which it appeared that the spread of the influenza bacillus infection from the upper to the lower respiratory tract permitted the ready entrance of secondary organisms into the lower respiratory passages. Cultures of the heart's blood remained

sterile except in one instance in which *Bacillus influenzae* was present in pure culture. The pathology of the disease is presented in the following paper.¹⁸ Illustrative protocols follow.

Experiment 11.—Monkey 20 (Text-fig. 5). *Macacus syrichtus*, male; weight 3,500 gm. Aug. 11 to 13, 1919. Well and active. Aug. 14, 10.30 a.m. Intratracheal injection of 5 cc. of broth suspension (five plates) of 12 hour culture of *B. influenzae* (Strain B. 11.8²). 4.15 p.m. Appears quite sick; resting against side of cage; breathing rapidly. 9.30 p.m. Very sick; lying down. Aug. 15. Sick; lying down or leaning against side of cage most of time; respirations moderately accelerated; frequent hard, racking cough. Aug. 16. Condition the same; cough continues. Aug. 17 and 18. Improving, but still sick and without appetite; appears weak and exhausted; coughs frequently. Aug. 19. Appears better and more active; breathing easily; no cough. Aug. 20. Appears well. 9.40 a.m. Killed.

Autopsy. Anatomical Diagnosis.—Acute tracheobronchitis; hyperplasia of thymus and thoracic lymph nodes.

Cultures.—Heart's blood, no growth; trachea, pure culture of *B. influenzae*.

Experiment 12.—Monkey 136 (Text-fig. 6, a). *Cebus capucinus* male; weight 1,100 gm. Aug. 14, 1919. Well and active. 2.30 p.m. Intratracheal injection of 1 cc. of peritoneal exudate of Passage Monkey 8, 10 minutes after removal of exudate at autopsy. 5 p.m. Begins to appear sick. 7 p.m. Very sick; lying prostrate; stuporous. 9 p.m. Prostrate; offers no resistance on handling; respirations accelerated. Aug. 15. Sick but no longer prostrated; no appetite; coughs. Aug. 16. Sick; lying down most of time; coughs occasionally. Aug. 17. Appears better but is weak and exhausted; no appetite; respirations moderately accelerated. Aug. 18. Condition the same. Aug. 19. More active. 11 a.m. Killed.

Autopsy. Anatomical Diagnosis.—Acute tracheobronchitis and bronchiolitis; hemorrhagic edema of lungs, all lobes; bronchopneumonia, all lobes; hyperplasia of thymus, spleen, and thoracic and abdominal lymph nodes.

Cultures.—Heart's blood and lungs, no growth; right bronchus, pure culture of *B. influenzae*.

Experiment 13.—Monkey 138 (Text-fig. 6, b). *Macacus syrichtus*, female; weight 2,915 gm. Aug. 16 to 18, 1919. Well and active. Aug. 19, 10.45 a.m. Intratracheal injection of 4 cc. of broth suspension (four plates) of 12 hour culture of *B. influenzae* (Strain B. 11.10²). 4.30 p.m. Sick; resting against side of cage; respirations very rapid. 7.30 p.m. Lying prostrate; groaning; coughs occasionally. Aug. 20. Prostrated; breathing rapidly; coughs. Aug. 21. Very sick. 10 a.m. Killed.

Autopsy. Anatomical Diagnosis.—Acute tracheobronchitis; lobular bronchopneumonia, right lower, left upper, and left lower lobes; acute pericarditis; chronic fibrous pleuritis, right side; hyperplasia of thymus and spleen.

Cultures.—Heart's blood, trachea, left lower lobe, and pericardium, pure culture of *B. influenza*.

TEXT-FIG. 6, *a* and *b*. Effect of intratracheal injection of *B. influenza*. (*a*) Monkey 136; tracheobronchitis, hemorrhagic edema of lungs, and bronchopneumonia. (*b*) Monkey 138; tracheobronchitis, bronchopneumonia, pericarditis, and septicemia.

DISCUSSION AND SUMMARY.

Twelve normal monkeys inoculated on the mucous membranes of the nose or nose and mouth with a strain of *Bacillus influenza* originally isolated in pure culture from the pleural exudate of a case of empyema following influenzal pneumonia in man and subsequently raised in virulence by animal passage, developed an acute self-limited

respiratory disease of from 3 to 5 days duration, characterized by sudden onset with profound prostration, the development of rhinitis and tracheobronchitis, with sneezing, cough, and the outpouring of a scanty mucoid, or mucopurulent exudate, a variable febrile reaction, and either a leucopenia or no significant change in the leucocyte count. This disease was complicated in five instances by purulent sinusitis of one or both antra, in three by bronchopneumonia. *Bacillus influenzae* was recovered at autopsy from the lesions of the disease either in pure culture or in association with organisms that are normal inhabitants of the upper respiratory tract of monkeys.

Of ten normal monkeys injected intratracheally with the same strain of *Bacillus influenzae*, seven developed bronchopneumonia, two developed tracheobronchitis without pneumonia, and one resisted infection. The general symptoms and duration of the disease were similar to those of the preceding group. There were a severe cough and accelerated respirations. *Bacillus influenzae* was recovered in pure culture from the lungs, bronchi, or trachea in the animals killed during the active stage of the disease. It disappeared promptly from the respiratory tract with recovery.

The significance of the first series of experiments in which monkeys were inoculated in the upper respiratory tract is twofold. First, they establish the fact that *Bacillus influenzae* can initiate in monkeys an acute infection of the normal mucous membranes of the upper respiratory tract; that is, it can act as a primary incitant of respiratory infection without the assistance of a preceding or concomitant contributing cause. In this respect it differs radically from the pneumococcus and *Streptococcus hæmolyticus*, since experiments previously reported^{2, 4} have shown that neither of these organisms possesses the property of initiating an infection of the normal mucous membranes of the upper respiratory tract of monkeys, even though the strains used were incalculably more virulent for monkeys than the strain of *Bacillus influenzae* used in the foregoing experiments. Secondly, the experiments show that *Bacillus influenzae* infection of the mucous membranes of the upper respiratory tract may spread by continuity to the paranasal sinuses, setting up an acute sinusitis, that it spreads readily to the lower respiratory tract, producing a tracheobronchitis and permitting the ready invasion of secondary bacteria, and that it may

penetrate as far as the terminal bronchioles, alveolar ducts, atria, and alveoli, there setting up a bronchiolitis and true bronchopneumonia. In these respects it likewise differs radically from the pneumococcus and *Streptococcus hæmolyticus* which do not possess these pathogenic properties as previous experiments have shown.^{2, 4}

The bearing of these facts on the possible etiologic relation of *Bacillus influenzae* to influenza is important, since they show that *Bacillus influenzae* possesses certain definite primary pathogenic properties which distinguish it and therefore separate it from the group of recognized secondary organisms in influenzal complications, of which the pneumococcus and the streptococcus are the most frequent. The possible etiologic relation of *Bacillus influenzae* to influenza is further supported by the character of the respiratory disease that occurred in the monkeys. The sudden onset with profound prostration, the absence of leucocytosis or often a leucopenia, the congestion of the mucous membranes of the respiratory tract, the development on the 2nd or 3rd day of an irritative cough due to an inflammatory tracheitis or tracheobronchitis, the brief self-limited course of the infection, and the irregular febrile reactions are all characteristic of influenza. Many of these symptoms were in striking contrast with the symptoms and course of pneumococcus or streptococcus infections in monkeys in which there were no prostration at onset, invariable leucocytosis, and infrequent cough developing only late in the disease.

While all the above features of the disease produced in monkeys are characteristic of influenza in man, none are pathognomonic and, in fact, it is doubtful whether uncomplicated influenza possesses any pathognomonic features by which it may be diagnosed certainly in the absence of an epidemic. Even during epidemic times many respiratory infections arise which, though presumably influenza, it is impossible to diagnose as such with certainty. Nor does pathology help in this respect, since there would appear to be no established distinctive lesions of uncomplicated influenza in man, nor for that matter of the complications of influenza, apart from the complications which have been ascribed by Pfeiffer,⁵ MacCallum,⁶ Wolbach,⁷ and others to infection with *Bacillus influenzae* because of the association of *Bacillus influenzae* in pure culture with these complications. For these reasons, although the disease produced in monkeys appears

to be essentially identical with influenza in man with respect to its clinical course and complications, it is impossible to determine certainly whether it is actually so. The experiments are advanced, therefore, as evidence in favor of the etiologic relation of *Bacillus influenzae* to influenza, though they do not permit of a definite conclusion in this respect.

Their bearing upon the relation of *Bacillus influenzae* to certain of the complications of influenza would appear to be reasonably conclusive. The recovery of *Bacillus influenzae* in pure culture at autopsy from the antra, from the trachea and bronchi, and from the lungs in some of the animals developing sinusitis, bronchiolitis, and a characteristic type of bronchopneumonia confirms by animal experiment the etiologic relation of *Bacillus influenzae* to these complications of influenza, which hitherto has rested solely upon the frequent association of the influenza bacillus with these lesions in man. The production of tracheobronchitis and the same type of bronchopneumonia by the intratracheal injection of *Bacillus influenzae* in the second series of experiments serves as additional confirmation of this, but has no direct bearing on the etiologic relation of *Bacillus influenzae* to uncomplicated influenza.

CONCLUSIONS.

1. *Bacillus influenzae* can initiate in normal monkeys an acute infection of the upper respiratory tract which may be complicated by acute sinusitis, tracheobronchitis, hemorrhagic edema of the lungs, bronchiolitis, and bronchopneumonia.

2. This disease appears to be essentially identical with influenza with respect to its clinical course, symptoms, and complications.

3. The etiologic relation of *Bacillus influenzae* to acute sinusitis, tracheobronchitis, bronchiolitis, and bronchopneumonia is established.

4. Although it seems reasonable to infer from the results of the experiments that *Bacillus influenzae* is the specific cause of influenza, a definite conclusion is not permissible, since it is impossible to determine whether the respiratory disease produced in monkeys with *Bacillus influenzae* is identical with influenza or merely similar to it. The experiments are advanced, therefore, as evidence in favor of the etiologic relation of *Bacillus influenzae* to influenza.