ACUTE RESPIRATORY INFECTION IN MAN FOLLOWING INOCULATION WITH VIRULENT BACILLUS INFLUENZAE

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The two epidemics of influenza which have swept over the country since the fall of 1918 have served to concentrate the attention of bacteriologists once more on the etiology of this disease, and a considerable amount of their interest has been centered on the part played by the influenza bacillus. During the last two years a number of investigators have tried to produce influenza in man by direct inoculation of B. influenzae cultures into the healthy nose and throat. Almost without exception, however, these efforts have resulted in failure.

Davis[1] succeeded in producing symptoms in a young man whose nose and throat he inoculated with a thick emulsion of influenza bacilli. Forty-eight hours after inoculation the patient complained of chilly sensations, headache and weakness; temperature 100.2, leukocytes 9,200. The throat was reddened and covered with mucus. The patient also developed a cough with mucopurulent expectoration. The acute symptoms lasted only 3 days. Cultures from nose, throat, and sputum showed many colonies of B. influenzae. The patient carried influenza bacilli in his throat for 4 weeks. The culture used by Davis was not isolated from a case of influenza, but from an uncomplicated case of pertussis.

Sellards and Sturm[2] inoculated human volunteers with a mixture of 5 strains of B. influenzae which had been isolated from cases of measles. These cultures had been under cultivation for 6-8 weeks before the tests were made. None of the volunteers developed any symptoms of measles or influenza, nor did they become carriers of influenza bacilli for any length of time.

Bloomfield[3] observed the effect of inoculating the nose and throat of human volunteers with strains of influenza bacilli isolated from the throats of healthy men. The 3 strains employed had all been under artificial cultivation for several weeks at the time of the experiments. Altogether 14 volunteers were tested. Four received the inoculation on the tongue; 5 in the nose; 3 on the tonsils; and 2 in the nasopharynx. None of the volunteers showed any symptoms, and none of them became carriers. In Bloomfield's experiments influenza bacilli had usually disappeared in cultures taken from the nose and throat 24 hours after inoculation.

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[1] Jour. Am. Med. Assn., 1919, 72, p. 1317.
[2] Bull. Johns Hop. Hosp., 1919, 30, p. 331.
[3] Bull. Johns Hop. Hosp., 1920, 31, p. 85.

A number of investigators have inoculated human volunteers with strains of B. influenzae isolated from cases of clinical influenza. Wahl, White and Lyall[4] inoculated volunteers with cultures of B. influenzae, spraying the nose and throat with an emulsion washed from a plate of chocolate agar. First the authors used old cultures, but as no results were obtained, the experiment was repeated four days later on two of the same volunteers with a freshly isolated strain (second generation). Of the two volunteers who received the fresh culture, one had no symptoms, while the other experienced only a slight local reaction in the nose on the afternoon of the injection. In these experiments, influenza bacilli persisted in the throats of the volunteers for 1-3 weeks or even longer.

The United States Public Health Service in conjunction with the U. S. Navy Medical Department carried out some interesting experiments with the influenza bacillus just after the epidemic of 1918. In Boston, M. J. Rosenau and his co-workers[5] injected a freshly isolated culture of B. influenzae into the nostrils of 6 volunteers, 3 of whom had a history of recent influenza and 3 of whom had no such history; none of the 6 developed symptoms. At the same time in San Francisco, McCoy and Richey[6] inoculated 5 volunteers with a heavy suspension composed of 8 strains of B. influenzae. Although none of this latter group of volunteers had had influenza, they all failed to show any symptoms following the inoculation.

From this review of the literature, it will be observed that, in the first place, the strains of influenza bacillus employed by these various investigators had, with two exceptions, been isolated from healthy individuals or from cases of measles or whooping cough. In the second place, the cultures used had usually been subjected to artificial cultivation for weeks or even months before the inoculations were made. The rapidity with which the influenza bacillus loses its virulence is well known. Finally, it has been shown that strains of influenza bacilli, like those of pneumococci, streptococci and other bacteria, differ greatly in their virulence. Some strains are entirely avirulent.

Blake and Cecil[7] recently have shown that by inoculating the nose and throat of monkeys with a virulent culture of B. influenzae, an acute infection of the upper respiratory tract may be induced, which is quite similar to influenza in man. In these experiments, the strain of influenza bacillus employed was first rendered virulent for the species by repeated intraperitoneal injections. By this procedure, the culture eventually became so virulent for monkeys that inoculation of the nose and throat with only a small quantity of either the peritoneal exudate or a blood broth culture would initiate an acute respiratory infection

[4] Jour. Infect. Dis., 1919, 25, p. 419.
[5] U. S. Public Health Rep., 1919, 34, p. 33.
[6] U. S. Public Health Rep., 1919, 34, p. 33.
[7] Jour. Exper. Med., 1920, 32, p. 691.

characterized by sneezing, coughing, mucopurulent discharge, leukopenia, prostration and fever. Furthermore, several of the monkeys thus infected later developed a hemorrhagic bronchopneumonia from which B. influenzae was recovered at necropsy.

If monkeys could be infected so readily with a virulent strain of B. influenzae, it seemed reasonable to suppose that, by use of proper methods, a similar experimental disease could be produced in man. The experiments reported in this paper were undertaken with such an object in view.

METHODS

Culture Mediums.—The culture mediums consisted of freshly prepared "chocolate blood" agar or broth, and the sodium oleate hemoglobin agar of Avery. The chocolate medium was prepared by adding defibrinated horse or rabbit blood to veal-infusion agar or broth, and the mixture heated at 70 C. for a few moments until the blood changed color. The reaction of the medium was P_H 7.4 to 7.6

Cultures.—Two strains of B. influenzae were employed in these experiments. Both were isolated in almost pure culture from patients with typical cases of influenza admitted to the Willard Parker Hospital during the epidemic of February, 1920. These two strains presented all the morphologic and cultural characteristics of the Pfeiffer bacillus and failed to grow on blood-free mediums.

Strain "Graham" was cultivated from the throat of a child with influenza and influenzal pneumonia. This strain was used in only one experiment. As the results were negative, it was discarded and another culture (strain "Wick") was substituted for the remaining experiments.

Strain "Wick" was isolated by Dr. A. W. Williams in conjunction with Streptococcus hemolyticus from the pleural exudate of a young woman who died of influenzal pneumonia and empyema. This strain was found to be virulent for both rabbits and mice. A rabbit injected intraperitoneally with 2 c c of a young chocolate blood broth culture died in 5 hours, and 0.25 c c of an 18-hour culture of the same strain killed a mouse in less than 24 hours.

Volunteers.—The volunteers selected for these experiments were healthy adults, either medical students or laboratory workers. Of the 6 persons inoculated with B. influenzae 3 gave a history of influenza during the epidemic of 1918-19. The other 3 denied having had the disease. Preliminary cultures were taken in every case from the nose

and throat to eliminate influenza bacillus carriers. One carrier, however, was purposely inoculated in order to determine whether the presence of influenza bacilli in the nose and throat conferred immunity against experimental infection. No volunteers were accepted who gave a history of any recent respiratory infection.

In addition to the 6 volunteers inoculated with influenza bacilli, 6 other volunteers were selected for control inoculations. Two of these received the filtrate from a chocolate blood broth culture of B. influenzae; 2 were inoculated with a virulent Streptococcus hemolyticus and the remaining 2 were tested with a group IV pneumococcus, which was highly virulent for mice.

All volunteers were kept under close observation during the period of the experiment. Temperature, leukocyte counts and cultures were frequently taken, and the subjects were carefully examined twice a day for any local or general reaction. When the influenza bacillus was recovered from cultures taken from the volunteers subsequent to inoculation, the strain was studied by means of agglutination and absorption tests to establish its identity with the strain inoculated. These tests were carried out by Dr. Olga R. Povitzsky of the Research Laboratory of the New York City Department of Health in connection with a biologic study of influenza bacilli undertaken by her simultaneously with the experiments herewith reported. Dr. Povitzsky's results are reported in detail in the Journal of Immunology for January, 1921.

INOCULATION OF VOLUNTEERS WITH WASHINGS FROM CHOCOLATE BLOOD-AGAR CULTURES OF BACILLUS INFLUENZAE

The nose and throat of two healthy volunteers were inoculated with freshly isolated strains of B. influenzae, which had been cultivated for 18 hours on chocolate blood agar slants. The growth was washed from each slant with 1 c c of sterile broth, and made a fairly thick suspension. One of these volunteers, (case 1) gave a history of influenza during the epidemic of 1918; the other (case 2) denied having ever had the disease. Preliminary cultures showed that neither of these volunteers carried influenza bacilli.

CASE 1.—L. B., a woman aged 28, had never had pneumonia; influenza in Oct., 1918. Rarely had colds. Last cold in Nov., 1919. Had never received influenza bacillus vaccine. No recent exposure to influenza. Well nourished; blond; throat and tonsils normal; slight deviation of nasal septum to right.

Feb. 14, 1920: Preliminary cultures from nose and throat on sodium oleate-agar plates show colonies of streptococci, staphylococci and diphtheroid bacilli; no influenza bacilli. Feb. 16, 11 a. m.: Received in each nostril one-half slant, in 0.5 c c of broth, of an 18-hour chocolate blood-agar culture of B. influenzae, strain "Graham." The culture used was the second generation on artificial medium. Most of the suspension trickled back into the nasopharynx and was spit out. At 2 p. m.: Complained of frontal headache; 5 p. m., headache severe. No reaction in nose or throat. Temperature normal. Right Nostril: Smears showed mucus, a few pus cells and intracellular influenza bacilli. Cultures showed staphylococcus albus and diphtheroid bacillus; no influenza bacilli. Left Nostril: smears, no pus cells, no influenza bacilli; cultures same as right nostril. Throat smears negative. Cultures: staphylococcus albus, streptococcus and a gram-negative diplococcus. No colonies of B. influenzae.

Feb. 17, 10:30 a. m.: Had a comfortable night. Awoke in the morning feeling well except for a slight rawness in the throat and some obstruction in the nose. 5 p. m.: She had noticed slight malaise all day.

Feb. 18: Malaise continued; no other symptoms.

Feb. 19: She felt slight malaise.

Feb. 20: She felt well.

In this case one-half slant of influenza bacillus culture produced practically no local symptoms. There was slight malaise for several days following the inoculation and a frontal headache, which lasted only 24 hours. Influenza bacilli disappeared from the nose and throat in a remarkably short time. Cultures taken 6 hours after inoculation were entirely free from influenza bacilli.

The next volunteer, case 2, received an entire chocolate-agar slant of B. influenzae culture in each nostril. A different strain (Strain "Wick") was employed for the inoculation (table 1).

CASE 2.—C. A. S., a man, aged 24, had never had influenza, though frequently exposed during the epidemic of 1918. No history of pneumonia. He had never received influenza vaccine. Last cold in Dec., 1919. He had no respiratory infection and had not been exposed recently to influenza. Physical examination revealed a well developed young man; his nose and throat were normal.

Feb. 10, 1920: Preliminary cultures from the nose and throat on blood-agar and chocolate blood-agar plates showed a predominance of Staphylococcus aureus and albus. Numerous colonies of Streptococcus viridans and a few colonies of a large gram-negative bacillus; no colonies of B. influenzae.

Feb. 12, 11 a. m.: The patient received, in each nostril, one entire slant (in 1 c c of broth) of an 18-hour chocolate blood-agar culture of B. influenzae, strain "Wick." The culture used was the third generation on artificial culture. A small quantity of the fluid passed back into the nasopharynx. 1:30 p. m.: The patient complained of headache and a burning sensation in the nose, accompanied by considerable serous discharge from both nostrils. Smears from nasal cavities taken at this time showed many influenza-like

TABLE 1

INOCULATIONS WITH CULTURES AND FILTRATES OF B. INFLUENZAE AND WITH STREPTOCOCCUS AND PNEUMOCOCCUS CULTURES

Volunteer	Age	History of Influenza	Dose in Each Nostril	Symptoms	Duration of Symptoms	Microscopic and Bacteriologic Results
1. M. B.	28	Fall of 1918	One half slant of culture of influenza bacillus	Headache, rawness throat, nasal obstruction, malaise	3 days	Smears: mucus and a few pus cells. Cultures: no influenza bacillus 6 hours after inoculation
2. C. A. S.	24	None	One slant of influenza bacillus	Headache, rhinitis, pharyngitis, malaise	2 days	Smears: many pus cells and influenza bacilli. Cultures: positive after 8 hrs., negative after 24 hrs.
3. E. M. C.	28	Fall of 1918	0.1 c c exudate	Headache, rhinitis, malaise, pharyngitis, tracheitis, backache, soreness in chest, diplopia	10 days	Many pus cells, many influenza bacilli; bacilli in cultures 2 weeks after inoculation
4. F. J. L.	47	Fall of 1918	0.1 c c exudate	Rhinitis, pharyngitis, conjunctivitis, headache, malaise, general muscular aching	3 days	Mucus, pus cells, a few influenza bacilli which were present in cultures 10 days after inoculation
5. W. J. S.	39	None	0.5 c c 6-hour chocolate brown culture of influenza bacillus	Headache, rhinitis, pharyngitis, conjunctivitis, tracheitis, backache, pain in legs	4 days	Many pus cells, many influenza bacilli in smears and cultures 5 days after inoculation
6. T. B.	32	None Influenza bacillus carrier	0.5 c c 6-hour chocolate brown culture of influenza bacillus	Rhinitis, malaise, headache, pharyngitis, tracheitis, soreness in chest	3 days	Many pus cells, many influenza bacilli in smears and cultures after inoculation
7. F. K.	21	None	0.5 c c filtrate 6-hour chocolate-broth culture of influenza bacillus	None		
8. M. K.	25	None	0.5 c c filtrate 6-hour chocolate-broth culture of influenza bacillus	None		
9. R. S.	21	None	0.5 c c 6-hour culture of streptococcus hemolyticus	Slight dryness and redness of throat	A few hours	Streptococcus hemolyticus in cultures 6 days after inoculation
10. H. W.	20	None	0.5 c c 6-hour culture of streptococcus hemolyticus	Headache, fever, leukocytosis, sore throat, exudate on tonsils	3 days	Streptococcus hemolyticus in cultures 4 days after inoculation
11. H. Z.	20	None	0.5 c c 6-hour culture of pneumococcus 4	None	.....	Pneumococcus 4 in cultures 48 hours after inoculation
12. C. W.	29	None	0.5 c c 6-hour culture of pneumococcus 4	Slight dryness in throat on third day	.....	Pneumococcus 4 in cultures 4 days after inoculation

bacilli. 3:30 p. m.: He complained of general malaise and drowsiness. 6:30 p. m.: The drowsiness was marked. He complained of rawness in throat and tender glands at angles of the jaw. There was a small amount of blood in the serous discharge from the left nostril and marked anorexia. The patient looked pale and sick; the nasal mucous membrane was swollen and reddened, and there was considerable serous discharge; the throat was intensely reddened and congested. The temperature was 97; pulse, 96. Smears from nasal cavities showed many pus cells and a few influenza-like bacilli, mostly intracellular. Cultures from the throat contained a few colonies of B. influenzae and a few colonies of a diphtheroid bacillus; from nasal cavities, no colonies of B. influenzae.

Feb. 13, 11 a. m.: Patient complained of headache and dizziness. There was complete obstruction of both nasal cavities early in the morning and considerable obstruction still existed. The patient had developed a dry, hacking cough. He woke up once during the night with burning and watering of the eyes. The nasal discharge was still abundant and the patient had to blow his nose frequently. Anorexia and general malaise persisted. The pallor was quite noticeable. The patient presented the picture of a mild influenzal attack; the mucous membrane of nose was still red and swollen; throat congested and covered with mucus. Smears from right nasal cavity showed a moderate number of pus cells but no influenza bacilli. Smears from the left nasal cavity showed many pus cells and a few influenza bacilli, both intracellular and extracellular. Cultures right nasal cavity, showed no colonies of B. influenzae; a few colonies of staphylococcus albus; from left nasal cavity no colonies of B. influenzae. There were a few colonies of a gram-negative diplococcus and a few colonies of a diphtheroid bacillus. Cultures from the throat showed many gram-negative diplococci and staphylococci. There were no B. influenzae colonies on the plate. 4 p. m.: Patient complained of feeling very tired. Temperature, 98 degrees. Sent home to bed.

Feb. 14, 10:30 a. m.: Patient felt better. Moderate amount of secretion still present in nasal cavity. Smears from nasal cavity showed mucus, desquamated epithelium and a few pus cells; no influenza bacilli.

Feb. 15: Patient felt well.

This volunteer appears to have been definitely infected by the inoculation. The local and constitutional symptoms were quite definite, and influenza bacilli were present in smears from the nostrils 24 hours after the injection of the culture. The temperature and leukocytes were unchanged. The most striking features were headache and general malaise, and the well marked local inflammation in the nose and throat.

From these two experiments it would appear that an acute but rather mild respiratory infection may be produced by the inoculation of chocolate blood-agar slant cultures of B. influenzae, if a sufficiently large dose of culture is employed. In view of the rapid disappearance of influenza bacilli in these two cases, the possibility of the reactions being of a toxic, rather than an infectious, nature cannot be excluded.

INOCULATION OF VOLUNTEERS WITH THE PERITONEAL EXUDATE FROM A MONKEY WITH BACILLUS INFLUENZAE PERITONITIS

Blake and Cecil [7] were successful in producing an acute respiratory disease in monkeys by direct inoculation with the peritoneal exudate from a monkey with B. influenzae peritonitis. It seemed desirable, therefore, to try this method of experimental infection on human volunteers. The exudate was obtained by inoculating a rhesus monkey intraperitoneally with a large amount (20 plates) of influenza bacillus culture. Twenty-four hours later the monkey was dead. The cloudy exudate, containing large numbers of influenza bacilli in pure culture, was removed from the peritoneal cavity with a sterile pipet and was injected immediately into the nostrils of the volunteers.

Two volunteers were inoculated with peritoneal exudate. The dose in both cases was quite small, only 0.1 c c of exudate in each nostril. In addition, the throats of the volunteers were rubbed with a cotton swab which had been soaked in the exudate.

CASE 3.—E. M. C., a man, aged 28, not susceptible to respiratory infections, had never had pneumonia. He had had a mild attack of influenza in the fall of 1918. He had had a mild cold in Oct., 1919. Free from cold and cough at present. Physical examination showed a well developed young man. Nasal passages clear. Mild chronic pharyngitis.

April 12, 1920: Preliminary cultures from nasal cavities showed staphylococcus albus. No colonies of B. influenzae on any of the plates.

April 13, 10 a. m.: Received 0.1 c c of B. influenzae exudate (strain "Wick") in each nostril, and throat was swabbed with B. influenzae exudate. 4 p. m.: Patient complained of slight headache and was sneezing. No malaise. Cultures from nasal cavities showed a few colonies of B. influenzae, and a few colonies of staphylococcus albus. The B. influenzae strain was biologically identical with strain "Wick." Culture from throat showed chiefly streptococcus viridans.

April 14, 10 a. m.: Comfortable night. In the morning there were no constitutional symptoms, but the patient complained of stuffiness in the nose. Smears from nose and throat were negative. Cultures from left nostril showed a few colonies of B. influenzae and staphylococcus albus. Cultures from right nostril showed a predominance of B. influenzae. Throat: streptococcus, Staphylococcus albus and B. influenzae.

April 15, 10 a. m.: General malaise; patient felt lazy and tired. Obstruction in nasal cavities and watery discharge from nose, particularly the right side. Cultures from right nostril showed almost pure growth of B. influenzae; from the left nostril, a few colonies of B. influenzae and Staphylococcus albus. Throat: Moderate number of B. influenzae colonies together with usual mouth flora. The B. influenzae strain isolated was biologically identical with Strain "Wick."

April 16, 10 a. m.: Patient felt better, except for cold in the head. Nasal cavities were still stopped up. Cultures from nose and throat positive for B. influenzae.

April 19: Still had mild cold in head. Cultures from throat and right nostril were positive for B. influenzae.

April 20, 10 a. m.: Cold in head persisted. Yesterday patient had long automobile ride. Wore no overcoat. Today complained of a recurrence of general malaise.

April 21: Patient was sneezing and coughing frequently, with scanty expectoration; no pain or discomfort, but general malaise was marked. Temperature 98 F. Went to bed. Smears from right nostril showed pus and influenza bacilli. Cultures: Almost pure growth of B. influenzae; smears from left nostril showed pus and many influenza bacilli; cultures, same as on right. Cultures from throat, positive for influenza bacilli. Smears from sputum showed mucus and pus; no bacteria. Cultures showed a few colonies of B. influenzae. The B. influenzae strain isolated was biologically different from Strain "Wick."

April 22: In bed most of day. Felt weak and inert. Profuse watery secretion from nose; cough, with mucopurulent expectoration. Soreness of muscles of chest; severe frontal headache and backache; diplopia and heaviness of eyelids. Temperature 99.4 F. Smears from nasal cavities showed pus and influenza bacilli. Cultures: almost pure growth of B. influenzae. Cultures from throat positive for B. influenzae. Smears from sputum showed many pus cells and many influenza bacilli. Cultures from sputum showed streptococci, staphylococci and influenza bacilli (about 20% of colonies).

April 23: Had severe coughing spell during night. Profuse nasal discharge continued; headache not so severe. The patient was pale and looked sick; dark rings about the eyes. Nasal mucous membrane was congested. Nasal discharge sometimes contained blood. His throat was still markedly inflamed. Cultures from nasal cavities showed scanty growths; no B. influenzae colonies. Cultures from throat also showed a large number of colonies of Streptococcus hemolyticus.

April 24: Patient looked and felt much better. Still had cold in head and coughed occasionally. Slight stiffness in back persisted.

April 25: Condition practically normal.

April 27: Felt all right. Cultures from throat showed no colonies of B. influenzae.

May 5: Patient continued well. Cultures from throat showed no colonies of B. influenzae.

This case was particularly interesting on account of the relapse which the patient experienced one week following the inoculation. The mild infection which developed immediately after the inoculation of B. influenzae had about run its course when, following exposure to cold, the patient developed a much more severe respiratory infection which involved the nose and throat and trachea and which necessitated his going to bed. Agglutination and absorption tests carried out with the cultures of B. influenzae isolated during the relapse indicated that this strain was entirely distinct biologically from the strain which was originally inoculated, and which had been isolated from the nose and throat during the first mild infection. The temperature and leukocytes in this case showed practically no deviation from normal. There was

a slight elevation of temperature at the onset of the relapse, but this was only temporary. It should also be noted that during the relapse the hemolytic streptococcus made its appearance in the cultures from the throat.

The next volunteer, case 4, received the same dose of peritoneal exudate that the volunteer in case 3 received. In spite of the small quantity of fluid injected (0.1 c c in each nostril), he promptly developed a characteristic chain of symptoms, as the following protocol will show (table 1, text-fig. 1).

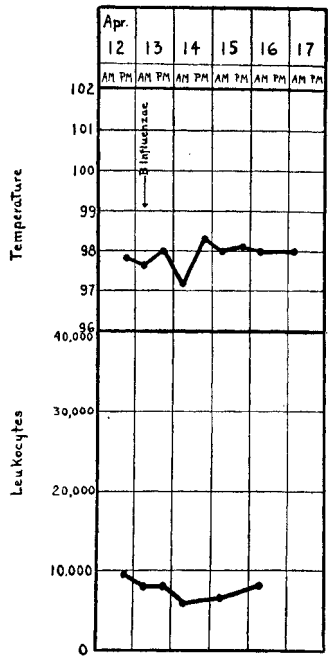

Fig. 1.—Case 4, F. L.; 0.1 c c B. influenzae exudate in each nostril.

CASE 4.—F. J. L., a man, aged 47, a laboratory technician, had influenza during the epidemic of 1918. There was no history of pneumonia; he rarely had a cold. He had had no colds at all during this fall and winter. A small swarthy man, well developed and well nourished. Pharynx and nasal passages normal.

April 12, 1920: Preliminary cultures from nostrils showed a few colonies of Staphylococcus albus and an occasional colony of a gram-negative diplococcus; no influenza bacilli. Cultures from throat; Streptococcus viridans, Staphylococcus albus and a gram-positive bacillus; no colonies of B. influenzae.

April 13, 11 a. m.: Received in each nostril, 0.1 c c of peritoneal exudate from a monkey with B. influenzae peritonitis (strain "Wick"). Throat rubbed with a cotton swab soaked in exudate. 1:30 p. m.: Nose began to run;

sneezed every few minutes; eyes were watering; throat slightly sore and raw. 4 p. m.: Patient complained of drowsiness and headache. Profuse serous discharge from nose. Nasal and pharyngeal mucous membrane swollen and reddened. Temperature 98 F. Cultures from left nostril were almost pure culture of B. influenzae. Throat culture showed a variety of bacteria; a few colonies of B. influenzae. Ten colonies of B. influenzae picked at random from plates were biologically identical with strain of B. influenzae inoculated.

April 14, 10 a. m.: Uncomfortable night, referable to headache, sore throat and copious nasal discharge. In the morning nose was stopped up and eyes were watering. He was still sneezing, but not so frequently. Complained of frontal headache and marked malaise. Patient presented the picture of a severe coryza. Mild conjunctivitis; mucous membrane of nose and throat reddened. Temperature, 97.2 F. Smears from the nasal cavities showed mucus, pus and a few influenza bacilli. Cultures from the nasal cavities gave almost pure growth of B. influenzae on both sides. The strain was biologically identical with strain "Wick." Cultures from throat yielded Streptococcus viridans, Staphylococcus albus, large gram-negative bacillus and a few colonies of B. influenzae. 4 p. m.: He went to bed on account of headache, dizziness and general malaise; complained also of pain in extremities, especially in elbows. Profuse diaphoresis; felt feverish. His temperature, however, was only 98 F.

April 15, 10 a. m.: Felt better, but malaise was still quite marked. Remained in bed a considerable part of the day. Not so much soreness in extremities, and headache was less severe. Nose was still discharging and both nasal cavities were obstructed. Cultures from right nostril showed Staphylococcus albus; left nostril, Staphylococcus albus and one colony of B. influenzae. Throat, usual mouth flora, and a few colonies of B. influenzae. Cultures from the conjunctiva showed Staphylococcus albus but no influenza bacilli.

April 16, 10 a. m.: Felt much better; returned to work. Nasal discharge much diminished. Smear from right nostril showed moderate amount of pus, no influenza bacilli. Cultures from right nostril showed Staphylococcus albus and a gram-positive bacillus; no influenza bacilli. Smears from left nostril showed pus cells and a few influenza bacilli. Cultures from left nostril showed no influenza bacilli. About 40% of the colonies in cultures from the throat were those of B. influenzae. Strain was biologically identical with "Wick" strain.

April 17: Condition was almost normal.

April 19: Patient felt all right. Cultures from right nostril were negative. Culture from left nostril showed 4 colonies of B. influenzae.

April 23: Culture from throat showed that 50% of colonies were B. influenzae. Strain was still biologically identical with "Wick" strain. Cultures from nostrils were negative.

May 5: Culture from throat showed 40% of B. influenzae colonies; strain identical with "Wick" strain.

This patient presented the picture of a mild, afebrile case of influenza. The prostration was quite marked and the headache and pains in the extremities were also characteristic. The leukocytes showed a definite leukopenia, the count going down from 9,300 to 6,000 (Fig.1). The temperature, as in the previous cases, remained normal; it was sub-

normal on the day following the inoculation. It is interesting to note that in this case influenza bacilli were still present in large numbers in the patient's throat four weeks after the inoculation.

INOCULATION OF VOLUNTEERS WITH YOUNG CHOCOLATE BLOOD-BROTH CULTURES OF B. INFLUENZAE

Two volunteers were inoculated with a 6-hour chocolate blood-broth culture of B. influenzae. The strain used for these inoculations was the same one used in the previous experiments with peritoneal exudate, but in this instance it was freshly isolated from the throat of the patient in Case 4. As in the two previous cases, very small quantities of culture were inoculated. These 2 volunteers each received 0.5 c c of culture in either nostril, but as the culture was very young, the actual number of bacteria injected was comparatively small. We were prompted to use the young cultures by the work of Parker,[8] who found that 6-hour cultures of B. influenzae were more toxic than older cultures.

CASE 5.—W. J. S., a man, aged 39; had never had influenza though frequently exposed. No history of pneumonia. Not susceptible to colds; had had only two mild colds during the past 6 years; no recent colds. A fairly well nourished man but rather poorly developed physically. Nose and throat normal.

April 19: Preliminary cultures from nose and throat showed normal mouth flora. No colonies of B. influenzae on any of the plates.

April 20, 10 a. m.: Received 0.5 c c of a 6-hour chocolate blood-broth culture of B. influenzae, strain "Wick," in either nostril. In addition, the throat was rubbed with a cotton swab soaked in a culture of the same organism. Culture used was the third generation on artificial culture. 12:30 p. m.: Complained of sharp headache. 3 p. m.: Sneezed frequently. Nose felt stopped up and there was a watery discharge from the nostrils. Throat was getting sore, and there was some pain in the right ear. In addition to these local symptoms there was general malaise, drowsiness, and aching in the shoulders. Patient looked moderately sick. Mucous membrane of nose and throat reddened. Smears from right nostril showed a few pus cells and intracellular bacilli. Cultures showed streptococci but no colonies of B. influenzae. Smears from left nostril were similar to those of right nostril. Cultures from left nostril showed no influenza bacilli. Culture from throat showed a few colonies of B. influenzae. Strain was biologically identical with strain "Wick."

April 21, 10 a. m.: Patient had a very uncomfortable night. Suffered from extreme exhaustion, severe frontal headache and insomnia. Locally there was obstruction in both nostrils, watery discharge from the nose, sore throat, burning of the eyes, tenderness of the glands in the neck, soreness in the chest and profuse diaphoresis. In the morning many of the symptoms persisted, and in addition patient had developed a hacking cough. He also complained of backache and pain down right leg. Patient looked pale and sick; throat congested, profuse nasal discharge. 4 p. m.: Patient had severe headache and backache, pain in the chest and cough with mucoid expectoration. Temperature 97.8 F. Smears from right nostril showed a few pus cells but no bacteria.

[8] Jour. Am. Med. Assn., 1919, 72, p. 476.

Culture from right nostril showed no influenza bacilli. Smear from left nostril showed many pus cells, gram-positive diplococci and gram-negative bacilli which stained poorly. Cultures negative for influenza bacilli. Culture from throat showed no influenza bacilli. Smears from sputum showed pus, mucus and many influenza bacilli. Culture from sputum showed a few colonies of B. influenzae.

April 22, 10 a. m.: Patient spent another restless night. This morning nose was still stopped up and throat sore. Complained of hoarseness and soreness in chest. Cough still present but not so severe. Headache and general malaise persisted. There was stiffness in the back when he attempted to bend over. Mucopurulent discharge from the nose. Patient still looked sick; rhinitis had improved, but throat was still red. Smear from right nostril showed many pus cells and a few intracellular influenza bacilli. Cultures

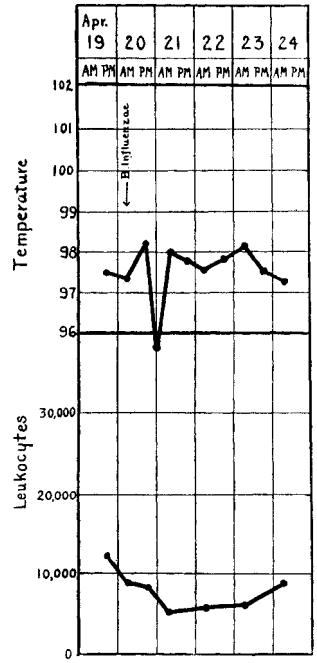

Fig. 2.—Case 5, W. J. S.; 0.5 c c 6-hour chocolate broth culture of B. influenzae in each nostril.

showed a few colonies of influenza bacilli. Smear from left nostril was similar to that of right. Cultures negative for influenza bacilli. Cultures from throat showed predominance of staphylococci, but 25% of colonies were those of B. influenzae. Strain biologically identical with "Wick" strain.

April 23: Patient was much better; had moderate rhinitis, no headache. Cultures from throat showed a great predominance of influenza bacillus (75%). Strain was biologically identical with "Wick" strain.

April 24: Patient's condition practically normal.

April 25: Patient was feeling well.

April 27: Patient remained well. Cultures from throat showed a large number of colonies of B. influenzae.

May 3: Cultures from throat still positive for B. influenzae. Patient felt well and throat appeared normal.

This volunteer reacted to the inoculation in a manner similar to that of the two preceding cases. The temperature was not elevated at any time but 12 hours after inoculation the temperature was 95.6. He also showed a stiking leukopenia, the number of leukocytes falling from 12,000 to 6,000 (Fig. 2). The patient became an influenza bacillus carrier following the inoculation. Cultures from the throat were positive for influenza bacilli for two or more months following inoculation.

Case 6 differed from the preceding cases in that the patient was a carrier of the influenza bacillus at the time of inoculation. It seemed desirable to inoculate one carrier purposely in order to determine whether the carrier state conferred immunity against infection by this organism. The method of inoculation was similar to that employed in case 5.

CASE 6.—T. B., a man, aged 32, had never had influenza though exposed many times. He had never had pneumonia and was not susceptible to colds. He had a mild coryza about one month before, the only respiratory infection he had had during the winter, and this attack lasted only two days. A small, muscular, robust young man; nose and throat normal.

April 19: Preliminary cultures from right nostril showed considerable number of B. influenzae colonies. Cultures from left nostril showed that B. influenzae was the predominating organism on the plates. The strain was biologically different from the "Wick" strain. Cultures from the throat showed usual mouth flora. A few colonies of B. influenzae were present.

April 20, 10:10 a. m.: Volunteer received 0.5 c c of a 6-hour chocolate blood-broth culture of B. influenzae, strain "Wick," in each nostril. The throat was rubbed with a cotton swab soaked in the same culture. Culture used was the third generation on artificial medium and had been isolated from the throat of the patient in case 4. 12:30 p. m.: Patient was sneezing. 4 p. m.: Nose felt stopped up and there were drowsiness and general malaise. On physical examination the patient appeared to be in good condition; throat negative. Smear from right nostril showed a few pus cells and intracellular influenza bacilli. Cultures showed predominance of B. influenzae. Smears from left nostril were similar to those from right. B. influenzae predominated in cultures. The strain was biologically different from the "Wick" strain. Cultures from throat were unsatisfactory, not suitable for examination.

April 21, 10 a. m.: Patient reported at laboratory. Had a rather uncomfortable night on account of sneezing, discharge from nose and burning of eyes. During the night he also developed a slight cough and felt sore through his chest. In the morning his chest was still sore and he had a cough with some expectoration. General malaise was marked, and he had pain in his chest when he coughed. Smears from each nostril showed mucus, many pus cells and a moderate number of influenza bacilli, some intracellular and some extracellular. B. influenzae predominated in cultures. Culture from throat

showed a variety of bacteria, but the colonies of B. influenzae were numerous. Smear from sputum showed mucus and many pus cells with moderate numbers of B. influenzae and a gram-positive diplococcus.

April 22, 10 a. m.: Patient had a headache the previous evening. Nose was still stopped up. Cough persisted but was not severe. Patient continued to feel tired and drowsy but remained on duty. Soreness in chest had disappeared. Smear from right nostril showed mucus and pus and many influenza bacilli. Gram-positive diplococci also were present. Cultures showed an almost pure growth of B. influenzae. Smear from left nostril showed pus and a few influenza bacilli. Cultures showed Staphylococcus albus and B. influenzae. In cultures from throat B. influenzae colonies predominated, constituting about 50% of all the colonies.

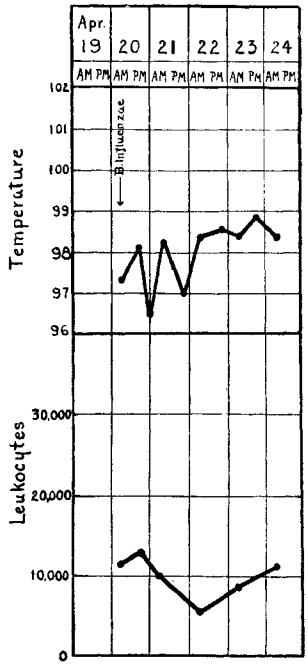

Fig. 3.—Case 6, T. B.; 0.5 c c 6-hour chocolate broth culture of B. influenzae in each nostril.

April 23: Patient was feeling well. Smears from right nostril showed pus and gram-positive diplococci. In cultures, B. influenzae predominated. Smears from left nostril were similar to those of right. Cultures were positive for B. influenzae (about 50% of colonies). Two types of influenza bacilli isolated from plates, one identical biologically with "Wick" strain, the other different. The two types were about equally represented.

April 27: Patient had entirely recovered. Culture from throat was positive for B. influenzae.

May 3: Culture from throat was positive for B. influenzae.

This patient ran a somewhat milder course than the other volunteers, and the question naturally arises whether the mildness of his

attack was dependent in any way on the fact that he was already an influenza bacillus carrier. Agglutination and absorption tests indicated clearly that the strain which he carried and the strain which was inoculated were entirely distinct biologically. It is possible, however, that there was some cross protection conferred by the strain which the volunteer carried. As in the previous cases, the temperature remained stationary and the leukocytes dropped; indeed, the temperature also dropped following the inoculation to 96.5 F. The leukocyte curve was quite similar to that observed in case 5. The patient's count before inoculation was 11,500. Following inoculation it gradually went down until it reached 6,500 on the second day of his infection (Fig. 3). This experiment would appear to indicate, however, that influenza bacillus carriers are not necessarily immune to infection by a different strain of influenza bacillus. Such a conclusion is in agreement with observations on carriers of certain other pathogenic bacteria.

INOCULATION OF VOLUNTEERS WITH FILTRATES OF BACILLUS INFLUENZAE CULTURES

In order to determine whether the symptoms produced by inoculation with B. influenzae were due to an infection or merely to the absorption of a toxic substance, two volunteers were inoculated with a filtrate from a young chocolate blood-broth culture of B. influenzae. After six hours' incubation the cultures were passed through a Berkefeld filter, and the filtrate inoculated at once into the volunteers. The same strain (strain "Wick") that was used for the preceding experiments was employed for the filtrate inoculations.

CASE 7.—F. K., a man, aged 21, had no history of influenza or pneumonia. He usually had one cold every winter; the last one in the autumn of 1919. Tonsils were removed in childhood. Healthy, well developed young man; nose and throat normal. Leukocytes 10,000.

May 2, 1920: Preliminary cultures from each nostril showed almost pure growth of staphylococcus albus. Cultures from throat showed pneumococcus type 4 and Streptococcus hemolyticus. No colonies of influenza bacillus on plates.

May 4, 3 p. m.: Received 0.5 c c of filtrate from a 6-hour chocolate blood-broth culture of B. influenzae, strain "Wick," in each nostril. The throat was rubbed with a cotton swab which had been soaked in the same filtrate. Culture used was the fourth generation on artificial medium.

May 5, 1 p. m.: Patient showed no symptoms following inoculation. Had noted a little phlegm in his throat, but smears taken from both nose and throat showed no pus cells present. Temperature 98.6 F.; leukocytes 10,700.

May 6, 1 p. m.: No symptoms except a slight headache early this morning, but he does not attribute it to the inoculation. (Subject to headaches, probably referable to eye strain.) Temperature 98.2 F.

May 7, 1 p. m.: No symptoms either local or general. Temperature 98.6 F.; leukocytes 11,000.

CASE 8.—M. K., a man, aged 25, a dental student, had no history of influenza or pneumonia; he was not susceptible to colds or sore throat. A healthy young man; nose and throat normal; leukocytes 7,000.

May 2, 1920: Preliminary cultures from nose and throat showed no colonies of B. influenzae on any of the plates.

May 4, 3 p. m.: Received 0.5 c c of a filtrate from a 6-hour chocolate blood-broth culture of B. influenzae, strain "Wick," in each nostril. In addition the throat was rubbed with a cotton swab soaked in the same filtrate. Culture used was the fourth generation on artificial medium.

May 5: Patient had developed no symptoms of any kind. Smears from nose and throat showed no pus cells present. Temperature 98.5 F., leukocytes 12,000.

May 6: No symptoms; nose and throat perfectly normal on examination; temperature 98.7 F.; leukocytes 9,300.

May 7, 1 p. m.; Patient remained well. Temperature 98.2 F.; leukocytes 11,200.

While it would be unwise to infer too much from only 2 experiments, it would appear from these experiments that filtrates of young cultures of B. influenzae are incapable of producing acute inflammatory symptoms in the nose and throat of healthy volunteers. It has been shown by Parker [8] that young cultures of B. influenzae contain a toxic substance, small doses of which will kill rabbits in a few hours. There was a possibility, therefore, that the symptoms produced by the intranasal injection of B. influenzae cultures were referable to absorption of this toxin. The negative results, however, obtained with filtrates from the cultures tend to eliminate such a possibility.

INOCULATION OF VOLUNTEERS WITH YOUNG BLOOD-BROTH CULTURES OF STREPTOCOCCUS HEMOLYTICUS

It seemed desirable to control the experiments with B. influenzae still further by inoculating some volunteers with other pathogenic bacteria. Accordingly, two volunteers who did not harbor Streptococcus hemolyticus in their noses or throats (2 colonies in throat of patient in case 9) were selected for inoculation with a strain of Streptococcus hemolyticus freshly isolated from the tonsils of a young woman with acute follicular tonsillitis and peritonsillar abscess. This organism was fairly virulent, 0.1 c c of a blood-broth culture killing a mouse in 24 hours.

CASE 9.—R. S., a man, aged 21, had no history of influenza or pneumonia. He had had two colds during the winter, the last one about two months before. He was subject to occasional attacks of sore throat but had never had ton-

sillitis. A healthy young man, well developed and well nourished; no nasal obstruction or inflammation; tonsils were large, otherwise throat was normal; leukocytes 11,000.

April 29: Preliminary cultures from right nostril showed that the predominating organisms were Streptococcus viridans and Staphylococcus albus; left nostril: Staphylococcus albus and a gram-negative diplococcus. Cultures from throat: The predominating colonies were pneumococcus type 4 (75% of colonies); staphylococcus colonies were fairly numerous; 2 colonies of Streptococcus hemolyticus.

May 1, 3 p. m.: Received 0.5 c c of a 6-hour chocolate blood-broth culture of Streptococcus hemolyticus in each nostril, and in addition the throat was rubbed with a cotton swab which had been soaked in the culture. This culture was the third generation on artificial medium (3 days after isolation from patient).

May 2, 10 a. m.: Patient had had no local or general symptoms of any kind. Temperature 98.2 F.; leukocytes, 9,600. Culture from right nostril showed pneumococcus type 4 and staphylococcus albus; no colonies of Streptococcus hemolyticus. Culture from left nostril was same as right except that the plate showed one colony of Streptococcus hemolyticus. Throat: At least one half of the colonies were those of Streptococcus hemolyticus.

May 3, 1 p. m.. Patient's throat felt a little dry in the morning when he first awoke, but after gargling this disappeared. Throat slightly red but tonsils showed no change. Temperature 97.9 F., leukocytes 11,300. Culture from each nostril showed that pneumococcus type 4 predominated; no colonies of Streptococcus hemolyticus were present. Culture from throat: Streptococcus hemolyticus predominated.

May 4: Nose and throat normal. Patient felt well. Temperature 97.4 F.; leukocytes 10,500. Cultures from nostrils were negative for Streptococcus hemolyticus. Culture from throat was positive for Streptococcus hemolyticus, but colonies were not so numerous as in culture of May 3.

May 5: Patient remained well. Temperature 98 F.; leukocytes, 10,200. Cultures from nostrils were negative for Streptococcus hemolyticus. Culture from throat showed that colonies of Streptococcus hemolyticus were still very numerous.

May 7: Culture from throat showed a few colonies of Streptococcus hemolyticus, but patient was entirely free from symptoms.

In this case the inoculation of a virulent Streptococcus hemolyticus gave practically negative results. The temperature and leukocytes remained normal. The patient had no constitutional symptoms, and locally the only change noticed was a slight dryness in the throat which lasted only a few hours. In spite of the failure, however, of the hemolytic streptococcus to produce infection, the organism remained in the patient's throat for a week, and perhaps longer, following the inoculation.

The second volunteer in this experiment (case 10) received the same strain of Streptococcus hemolyticus and the same amount of culture as case 9. The results, however, were more definite, as the following protocol will show.

CASE 10.—H. W., a man, aged 20, had no history of influenza or pneumonia. He had an average of about two colds a year. He had had his last cold five weeks before. He had never had tonsillitis and no nasal trouble. A robust, well nourished young man; nose and throat normal; tonsils small and appeared to be normal.

April 29, 1920: Preliminary cultures from nostrils showed staphylococcus albus. Culture from throat showed the usual mouth flora with Staphylococcus albus predominating; no colonies of Streptococcus hemolyticus on any of the plates.

May 1, 3 p. m.: Received 0.5 c c of a 6-hour chocolate blood-broth culture of Streptococcus hemolyticus in each nostril. Throat also rubbed with a cotton swab soaked in the same culture. The culture used was the third generation on artificial medium.

May 2: During the night the patient had a headache and was conscious of a slight soreness on the right side of the throat. The tonsils appeared somewhat reddened in the morning and there was still slight headache. Temperature 98.4 F.; leukocytes 10,000. Culture from right nostril showed 4 colonies of Streptococcus hemolyticus; Staphylococcus albus predominated. Cultures from left nostril showed pure growth of Staphylococcus albus. There were a few colonies of Streptococcus hemolyticus in the throat. Streptococcus viridans and Staphylococcus albus predominated.

May 3, 1 p. m.: Soreness in throat was more marked on left side. Felt well with the exception of a sore throat and slight headache. Temperature 100.6 F.; leukocytes 18,000. Cultures from nostrils were negative for Streptococcus hemolyticus. Culture from throat showed an increasing number of Streptococcus hemolyticus colonies.

May 4: Condition had improved, but throat was still sore. He did not sneeze or cough. Glands in neck felt a little sore. Tonsils were still inflamed and covered with a moderate amount of exudate. Cultures from nostrils were negative for Streptococcus hemolyticus. About 15% of colonies in cultures from throat were those of Streptococcus hemolyticus.

May 5: Patient felt well. Throat appeared normal. Cultures from nostrils were negative for Streptococcus hemolyticus. Three colonies of Streptococcus hemolyticus were found in throat culture.

May 7: Patient remained well.

It will be seen from this protocol that this volunteer developed an acute follicular tonsillitis following inoculation with Streptococcus hemolyticus. The infection, however, was a mild one and lasted only 3 days. There was a moderate febrile and leukocyte reaction and mucopurulent exudate over the tonsils. The patient was practically free from malaise. Headache was present, but not severe. Hemolytic streptococci persisted for 5 days, and perhaps longer, in the nasopharynx.

Summarizing these two experiments with the Streptococcus hemolyticus, it may be said that the inoculation of healthy volunteers with a virulent Streptococcus hemolyticus produced either no symptoms at all or gave rise to an acute tonsillitis with fever and leukocytosis.

INOCULATION OF VOLUNTEERS WITH A YOUNG BLOOD-BROTH CULTURE OF PNEUMOCOCCUS

As a further control on the B. influenzae inoculations, two volunteers were selected for inoculation with a virulent pneumococcus. For this experiment two healthy men were chosen whose preliminary cultures showed them to be free from pneumococci. A freshly isolated, highly virulent pneumococcus group 4, was used for these inoculations. This strain had been obtained from the sputum of a child with influenzal pneumonia.

CASE 11.—H. Z., a man, aged 20, gave no history of influenza or pneumonia. He had had frequent attacks of tonsillitis in childhood, but none since 1915. He has two or three colds every year; last cold about four months ago. A healthy, well developed young man; no nasal obstruction; throat clean; tonsils small; leukocytes 10,300.

April 29: Preliminary cultures from nostrils showed Staphylococcus albus and Streptococcus viridans; usual mouth flora; no pneumococcus colonies observed on any of the plates. Mouse inoculated intraperitoneally with 0.5 c c of patient's saliva remained well.

May 1, 3 p. m.: Received 0.5 c c of a 6-hour chocolate blood-broth culture of pneumococcus type 4, in each nostril, and throat rubbed with a cotton swab soaked in the same culture. Culture used was the third generation.

May 2, 10 a.m.: Patient sneezed twice on afternoon of May 1 after receiving the inoculation; otherwise he had had no symptoms, either local or general. Temperature 98.4 F.; leukocytes 9,900. Cultures from nostrils showed Staphylococcus albus; from throat, Streptococcus viridans, Staphylococcus albus and a gram-negative bacillus; no pneumococcus colonies on any of the plates.

May 3: He continued to feel well. Temperature 98.6 F.; leukocytes 9,900. Smears from nostrils were negative. Cultures from nostrils showed Staphylococcus, Streptococcus viridans and pneumococcus type 4. Cultures from throat showed Streptococcus viridans and Staphylococcus albus.

May 4: No symptoms; temperature 98 F.; leukocytes 10,200. Cultures from right nostril showed Staphylococcus albus; from the left nostril, Streptococcus viridans, Staphylococcus and gram-negative diplococcus.

May 5: Remained well. Temperature 98.8 F.; leukocytes 9,100. Cultures from nostrils were negative for pneumococcus.

May 6 to 8: Patient remained well.

This volunteer showed no reaction, either local or general, to inoculation with a pneumococcus group 4, though the culture inoculated was highly virulent for mice and had been freshly isolated from a case of influenzal pneumonia.

A second volunteer was inoculated with the same culture of pneumococcus and he, too, failed to develop any symptoms.

CASE 12.—C. W., a man, aged 29, gave no history of influenza or pneumonia. He was not subject to colds or sore throats; last cold about three months ago.

A well developed young man; throat clean, nasal passages clear; leukocytes 8,400.

April 29, 1920: Preliminary Cultures on Blood-Agar Plates: Nostrils: Staphylococcus albus; throat: Staphylococcus albus and Streptococcus viridans. White mouse inoculated intraperitoneally with one-half c c of saliva remained well.

May 1, 3 p. m.: Received 0.5 c c of a 6-hour blood-broth culture of pneumococcus type 4 in each nostril; in addition the throat was rubbed with a cotton swab soaked in the same culture.

May 2, 10 a. m.: Sneezed several times May 1 just after receiving the inoculation. Developed no symptoms last night. Felt well in the morning. Temperature 98.5 F.; leukocytes 10,000. Cultures from right nostril showed Staphylococcus albus and pneumococcus type 4; one colony of pneumococcus type 3. Left nostril: showed Staphylococcus albus and pneumococcus type 4. Throat showed about 50% of colonies are those of pneumococcus type 4.

May 3, 1 p. m.: Throat felt a little dry; otherwise no symptoms. Temperature 98.6 F.; leukocytes 13,700. Smears from nostrils showed no pus cells. Cultures from nostrils showed Staphylococcus albus; from throat, a few colonies of Streptococcus viridans, Staphylococcus albus and pneumococcus type 4.

May 4: No symptoms in respiratory tract. He had slight indigestion; temperature 98.7 F.; leukocytes 10,400. Culture from right nostril showed predominance of pneumococcus type 4 colonies; a few colonies of pneumococcus type 3. Left nostril was negative for pneumococcus. The plate showed chiefly Staphylococcus albus. The throat plate was overgrown with some saprophyte.

May 5: Felt well; temperature 98.2 F.; leukocytes 9,400. Cultures from nostrils were negative for pneumococcus. Cultures from the throat showed a few colonies of pneumococcus type 4.

May 8: Remained well.

The patient case 12, as in case 11, failed to develop symptoms of a respiratory infection following the inoculation of virulent pneumococci. The negative results obtained in these two volunteers indicate that in man the inoculation of virulent pneumococci in the healthy nose and throat is not sufficient in itself to induce a pneumococcus infection of the upper respiratory tract, though no sweeping conclusion can be based on so small an experiment.

DISCUSSION

The results obtained in these experiments confirm, in almost every particular, the studies on experimental influenza recently carried out by Blake and Cecil on monkeys. In both instances, the inoculation of virulent influenza bacilli usually gave rise to an acute respiratory disease, similar in many respects to clinical influenza, and characterized by the presence of influenza bacilli in the discharges. In monkeys the hemolytic streptococcus, when inoculated in the nose and throat, caused no reaction. In man, however, one of the two volunteers developed an acute tonsillitis from which Streptococcus hemolyticus was recovered.

In both species virulent pneumococci failed in a few trials to excite symptoms.

In view of the failure of most of the previous experimenters to produce a respiratory infection in man with the influenza bacillus, it is interesting to speculate as to the reasons for success in our own experiments. The use of freshly isolated, virulent strains was probably an important factor. The character of the culture medium employed also appeared to have considerable influence. The first experiment with the washings from chocolate blood-agar slants was practically negative. The second experiment with the same sort of suspension was fairly successful, but a large dose (one slant in each nostril) was employed, and the symptoms produced were temporary. The two experiments with small doses of peritoneal exudate from a monkey with B. influenzae peritonitis were successful; and so were the last two experiments with small doses of young chocolate blood-broth cultures. It would appear, therefore, that fluid cultures were more likely to produce positive results than the washings from solid medium.

As to the character of the infection produced by these inoculations, the most that can be said is that the experimental disease closely simulated the milder forms of endemic influenza or severe coryza. The short period of incubation in the experimental disease agrees with clinical observations as to the incubation period of influenza in some cases. In respect to the local manifestations, the analogy is striking. The onset with sneezing, rhinitis and sore throat, followed in some cases by tracheitis, presents a marked similarity to influenza. The systemic reaction in the experimental disease was not so profound as in true influenza, and resembled more the prostration that accompanies a severe cold or bronchitis. It may be remarked in passing, however, that many common colds are apparently due to the influenza bacillus. Headache was noted in 5 of the volunteers. The prostration in case 3 was quite marked, and the patients in cases 4 and 5 were in bed during part of their illness. Leukopenia was present in 3 of the cases (4, 5 and 6), in the other 3, the leukocytes remained practically unchanged. Here again the findings were in harmony with clinical influenza and with the experimental infections produced in monkeys. The absence of fever in all 6 cases was rather surprising. Its absence constituted a point of divergence from typical influenzal infections and throws some doubt on the identity of the experimental disease with influenza as seen in the

epidemics. The occurrence of severe influenza without a rise of temperature is not unusual, however. The patient with the fatal case of influenzal pneumonia from which strain "Wick" was isolated, and with which 5 of the volunteers were inoculated, had been in the hospital only 48 hours when death occurred. During that time, however, the temperature did not rise above 99° F.

The influenza bacillus was recovered from the nose and throat of the 5 volunteers that developed local symptoms. In case 2, however, the influenza bacilli had disappeared 24 hours after inoculation. In the 4 remaining cases (those that received B. influenzae exudate or blood-broth culture) influenza bacilli persisted in the nose or throat for several days or even weeks after inoculation. Many of the plate cultures taken at the height of the infection gave almost pure growths of B. influenzae.

Agglutination and absorption tests were carried out by Dr. Povitsky of the Research Laboratories on the strains recovered from the patients, and they proved in every case to be identical with the strain inoculated. Cases 3 and 6 are of special interest in that they throw some light on the question of B. influenzae immunity. The patient in Case 3 was making rapid recovery from his infection when, following exposure to cold, he had a relapse and was more ill than he had been during the first attack. Cultures taken during the first attack showed influenza bacilli identical biologically with the strain inoculated. When, however, cultures were taken during the relapse, influenza bacilli were recovered in large numbers, but agglutination and absorption tests showed that they were of a strain entirely different from the one originally injected. Apparently, infection with one strain of B. influenzae gave no protection against reinfection with another strain of different type. The patient in case 6 was an influenza bacillus carrier, but was purposely inoculated with strain "Wick" to find out whether he possessed any immunity against this organism. The strain he carried and the strain inoculated were biologically unlike. Following inoculation he developed practically the same chain of symptoms, both local and constitutional, that had been observed in the previous patients, none of whom had been B. influenzae carriers. Furthermore, when cultures were taken on the third day after inoculation, and a number of B. influenzae colonies were picked from the plates, 2 strains of B. influenzae were recovered, the strain which had been present in the throat before inoculation and the actual strain injected. One week after inoculation, strain "Wick" had disappeared entirely from the

nose and throat and the carrier strain only survived. The conclusion may be drawn that B. influenzae carriers are not necessarily immune to a virulent strain of this organism.

In some of the volunteers, the cultures remained positive for B. influenzae a surprisingly long time after recovery from the infection. In case 3, the cultures were still positive 2 weeks after inoculation. In case 4, positive cultures were obtained more than 2 months after inoculation. The identity of these strains with the strain inoculated was, of course, verified by agglutination and absorption tests. These results were in sharp contrast with those of Bloomfield, who found that strains of B. influenzae isolated from healthy mouths disappeared rapidly after injection into the healthy nose and throat.

The experiments with filtrates of B. influenzae cultures clearly eliminate the possibilty that the results obtained in these experiments might have been referable to some soluble toxic substance. Even admitting that a small portion of the toxin remained behind in the filter, this must have been an inconsiderable part of the whole, and not enough to have materially affected the result. There is abundant clinical and bacteriologic evidence that we were dealing in these cases with genuine infections.

The experiments with the hemolytic streptococcus are interesting and corroborate the findings of Richey,[9] who noted a number of cases of streptococcus hemolyticus tonsillitis following inoculation of healthy volunteers with the washings from the noses and throats of patients with influenza. It is probable that altogether, aside from the question of virulence, the results following inoculation with Streptococcus hemolyticus depend largely on the source of the strain. In this particular instance, a strain isolated from a case of acute tonsillitis with peritonsillar abscess, excited an attack of acute tonsillitis in one of the volunteers inoculated with the culture.

A virulent pneumococcus of the type 4 group isolated from a case of influenzal pneumonia, was inoculated into the nose and throat of two volunteers and failed to excite any symptoms. It should not be inferred from these two experiments, however, that the pneumococcus never acts as the primary infectious agent in acute infections of the nose and throat. It is possible that pneumococcus and certain others of the bacteria that are associated with coryzas and sore throats invade the tissue only after some depression in the local or general resistance of

[9] Jour. Infect. Dis., 1919, 25, p. 299.

the host. The recently published experiments of Grant, Mudd and Goldman [10] bear out this theory. The capacity of all these bacteria to act as secondary invaders cannot be questioned; but the experiments reported in this paper indicate that virulent influenza bacilli possess a peculiar power of attacking healthy mucous membrane and of exciting a respiratory infection quite similar in some of its clinical manifestations to spontaneous influenza.

SUMMARY AND CONCLUSIONS

Virulent influenza bacilli, when injected into the nose and throat of healthy volunteers, may excite in them an acute respiratory disease, similar in many respects to influenza, but falling short of the typical clinical picture.

In such cases influenza bacilli, biologically identical with those inoculated, may be recovered from the discharges as long as symptoms persist and often for some time thereafter.

Filtrates of B. influenzae cultures, when similarly injected into two healthy volunteers, produced neither local nor constitutional reaction.

The inoculation of healthy volunteers with virulent hemolytic streptococci may in some cases induce an acute follicular tonsillitis, with fever and leukocytosis. A virulent pneumococcus type 4 on the other hand, was injected into the nose and throat of two healthy volunteers with impunity.

[10] Jour. Exper. Med., 1920, 32, p. 87.