

EXPERIMENTAL STUDIES OF THE NASOPHARYNGEAL SECRETIONS FROM INFLUENZA PATIENTS.

III. STUDIES OF THE CONCURRENT INFECTIONS.

BY PETER K. OLITSKY, M.D., AND FREDERICK L. GATES, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

PLATE 35.

(Received for publication, December 13, 1920.)

In two previous communications^{1,2} the effects produced in the lungs and blood of rabbits through the intratracheal injection of the nasopharyngeal secretions from patients in the early stages of uncomplicated epidemic influenza were described and traced to a living substance not of the nature of ordinary bacteria. Nevertheless, ordinary bacteria were occasionally, if rarely, encountered in the lungs in the course of the experiments, in which case the lesions of the lungs and the composition of the blood were quite different from those observed in the absence of ordinary bacteria. Since we had in view a possible relation of the active substance giving rise to the peculiar effects observed in the lungs and the blood to the etiological agent of influenza, and since epidemic influenza in man so commonly predisposes to a variety of secondary pulmonary infections, it was also deemed advisable to study the concurrent ordinary bacterial invasions.

The subject was approached from two points of view. First, the circumstances under which ordinary bacteria accidentally invaded the lungs in the course of the transmission experiments were considered, and second, experimental concurrent infections were induced in order to imitate in the rabbit the operation of the predisposing influences of influenza leading to secondary pulmonary affections.

As previously stated, the unfiltered¹ or filtered² nasopharyngeal secretions of early cases of epidemic influenza were injected into the

¹ Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 125.

² Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 361.

lungs of rabbits by way of the trachea.³ With unfiltered secretions bacteria present in the nose and throat would, of course, be carried into the lungs; but the bacteria often disappeared or were eliminated in course of subsequent transmission experiments. The kinds of bacteria and the number of times they were observed in the various rabbit and guinea pig passages were as follows:

Pneumococcus Type IV.....	11
“ “ II atypical.....	3
Gram-negative, hemoglobinophilic bacillus.....	2
<i>B. pyocyaneus</i>	2
“ <i>bronchisepticus</i>	2
<i>Micrococcus catarrhalis</i>	1
<i>B. coli communior</i>	1
<i>Streptococcus viridans</i>	1
“ <i>hemolyticus</i>	1
Gram-negative, slender, spore-bearing bacillus.....	1
Streptothrix.....	1

There was no regularity in incidence or species of the microorganisms found in occasional rabbit passages of a consecutive series. For example, in the filtrate series of ten successive passages from Case 17,¹ the fifth passage yielded Pneumococcus Type IV, the seventh and eighth passages *Bacillus pyocyaneus*,⁴ and the tenth passage a small, Gram-negative, hemoglobinophilic bacillus. The filtrate series of five such passages of glycerolated material derived from Patient 26 yielded a Gram-negative, long, slender, spore-bearing bacillus in the fifth passage, while the glycerolated series of ten passages of material from Case 17 yielded in the tenth passage *Bacillus coli communior* and a streptothrix. All the remaining passages in these various series were sterile in the ordinary sense. Obviously, therefore, because of this variety the infections may be regarded as of accidental nature. In general these bacteria appeared more often in the earlier passages of unfiltered, and in the later passages of filtered material.

That the appearance of the ordinary bacteria was accidental is shown by single instances of bacterial infection in several series of

³ All operations were performed under light ether anesthesia.

⁴ The occurrence of *B. pyocyaneus* was the result of faulty technique in filtration.²

consecutive animal passages otherwise free. Thus in the glycerolated series of Case 17 only the tenth passage yielded *Bacillus coli communior* and a streptothrix, while the filtrate in the same series showed in the fifth passage only Pneumococcus Type IV. Again the glycerolated filtrate series from Patient 26 exhibited in the fifth passage a Gram-negative, spore-bearing bacillus, and the unfiltered nasopharyngeal washings from Case 11 yielded in the third passage Pneumococcus Type IV.

In the instances in which aerobic bacteria were encountered in the first passages, the suppression of the bacteria, as already alluded to, could often be effected so that the subsequent passages were free from them. Thus in the transmissions of the nasopharyngeal secretions from Patient 6 the first passage showed Pneumococcus Type IV, while in the second, third, and fourth passages no aerobic microorganisms were found. When, therefore, the "influenzal agent," if it may be so designated for purposes of clearness, was present, its peculiar effects could be recognized in the lungs and blood. The suppression of ordinary bacteria was effected as follows: The ordinary bacteria tended to induce consolidation and even abscess of the lung and often remained localized in these areas. The influenzal agent tended to diffuse throughout the lung tissue. By killing the animals early, portions of the lungs could be selected which were free from ordinary bacteria and yet contained the active agent in a transmissible state. Of the seven patients whose nasopharyngeal secretions gave rise to typical effects in rabbits, in four the ordinary bacteria present in the washings were suppressed completely in this manner so that all the animals inoculated subsequently remained free from them.

The clinical condition of the rabbits developing concurrent infections tended to be more grave than that of rabbits in which this form of infection was avoided. The conjunctivitis was purulent instead of catarrhal. There was more loss of weight, and often the animals were prostrated and died in 36 to 48 hours.

The blood showed a greater depression of the leucocytes, involving the polymorphonuclear cells and especially the mononuclear cells in the animals which succumbed acutely; in the others an initial depression was followed in 24 to 48 hours by a leucocytosis, usually a polymorphonucleosis.

The pathological appearances also differed. Whereas in the absence of ordinary bacteria the lungs showed hemorrhagic edema and emphysema, without consolidation and pleuritis, in the presence of these bacteria a pneumonic consolidation involving one or more lobes and sometimes actual abscess with softening of the tissues arose. The several kinds of bacteria mentioned were found more or less abundantly in the consolidated areas.

It is obvious, therefore, that the effects of materials derived from cases of influenza in man on the lungs and the blood of rabbits are sharply distinguished according as common kinds of bacteria do or do not multiply in the lungs. Under the former circumstances the ordinary and usually severe inflammatory reaction follows; under the latter conditions a peculiar hemorrhagic edema of patchy character arises in the lungs, and the blood shows a marked and characteristic leucopenia with mononuclear depression.

Experimental Concurrent Infection.

It has been commonly and widely noted that cases of influenza in man, uncomplicated by concurrent infections of the bronchi and lungs, tend quickly to recover, but that when these structures are involved in ordinary bacterial infections several kinds of bacteria appear, among which are streptococci, Pfeiffer bacilli, pneumococci, staphylococci, and, rarely, meningococci and even other bacteria; in this event the disease process is correspondingly rendered more intense. The impression is widespread that the inciting agent of influenza, whatever it may be, renders the lung structures more vulnerable to these bacteria, many of which are ordinarily present in the nasopharynx in health. Hence the question at once arose whether the influenzal agent under consideration also predisposed the pulmonary structures to such concurrent or secondary infections. A series of experiments was devised to test this point. The first step was to determine the effects of pure cultures of the several bacteria alone.

Intratracheal Inoculation of Bacteria.

The microorganisms of the ordinary kind to which attention was directed were a Type IV and an atypical Type II pneumococcus, *Bacillus pfeifferi*, and *Bacillus bronchisepticus*, the last because it is a common inhabitant of the upper air passages of the rabbit.

Pneumococcus.—The strains of pneumococci employed were freshly isolated from the lungs of rabbits used in the transmission experiments, or directly from the nasopharyngeal washings and sputum from the secondary pneumonias of cases of influenza, or from the normal sputum of man.

If small numbers of the pneumococci were inoculated intratracheally in rabbits, the blood picture remained unchanged for 2 to 4 days, after which a more or less pronounced polymorphonuclear leucocytosis arose which lasted for 2 to 4 days, when the blood returned to normal. Larger doses of the pneumococci led to a prompt polymorphonucleosis, a bacterial invasion of the blood stream, and death. The lesions present in the lungs and pleura consisted of consolidation and exudate similar to that described by Lamar and Meltzer⁵ and Wollstein and Meltzer.⁶ Filtrates from these consolidated lung tissues were without pronounced effect on normal rabbits when injected intratracheally.

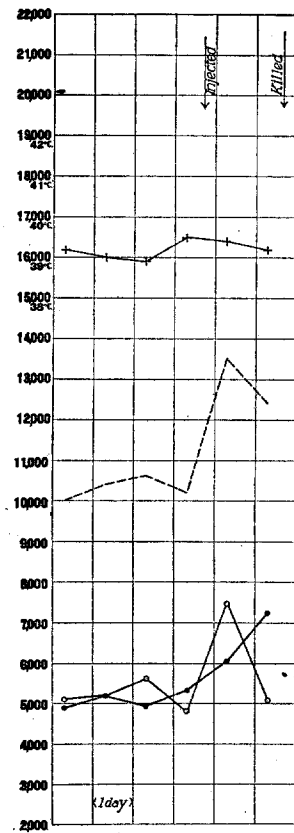
B. pfeifferi.—The *B. pfeifferi* used for inoculation was obtained from lungs of fatal cases of influenza, from exploratory thoracentesis, and from the sputum of cases of influenza. *B. pfeifferi* were suspended in saline solution (12 to 60 billion per inoculation) and injected intratracheally. Three separate strains were injected. The passage of one of the strains through two rabbits in succession and of the other two through three rabbits was attempted. The secondary passages were made with ground and suspended lung tissues of the rabbits previously inoculated. The effect of the injections was to induce a leucocytosis which was generally of a polymorphonuclear type and was still present at the end of 48 hours when the animals were killed in order to observe the lung lesions (Text-figs. 1 to 3). Two of the strains induced no visible lung lesions; the third strain seemed to set up a pneumonic consolidation from which, however, not *B. pfeifferi* but an atypical Pneumococcus Type II was isolated. In no instance was *B. pfeifferi* recovered from the injected lungs.

The toxic extract described by Parker⁷ was injected into the lungs of seven rabbits in doses of from 2 to 3 cc. In certain rabbits a polymorphonucleosis was set up, in others no blood changes were produced (Text-fig. 4). No visible pulmonary lesions resulted.

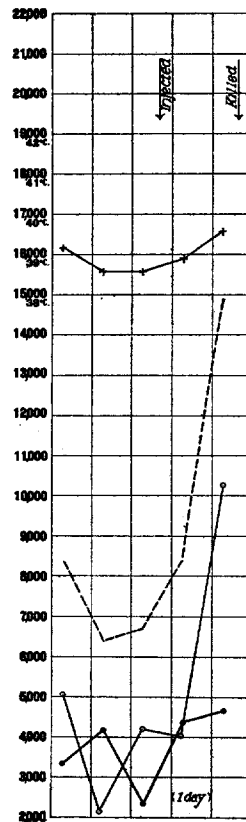
⁵ Lamar, R. V., and Meltzer, S. J., *J. Exp. Med.*, 1912, xv, 133.

⁶ Wollstein, M., and Meltzer, S. J., *J. Exp. Med.*, 1913, xvii, 353.

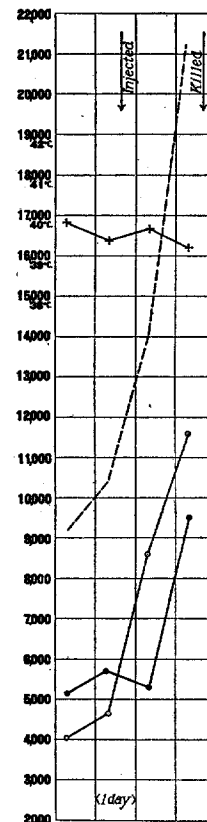
⁷ Parker, J. T., *J. Immunol.*, 1919, iv, 331.



TEXT-FIG. 1.



TEXT-FIG. 2.



TEXT-FIG. 3.

—•— Temperature
 - - - Total leucocytic count
 —▲— Mononuclears
 —●— Polymorphonuclears

TEXT-FIG. 1. Effect on the blood count and temperature of the intratracheal inoculation of *B. Pfeifferi*. First rabbit passage. The leucocytic rise, the polymorphonucleosis, and the variable temperature reaction are noteworthy.

TEXT-FIG. 2. Effect on the blood count and temperature of the intratracheal inoculation of *B. Pfeifferi*. Second rabbit passage. The leucocytic rise, the polymorphonucleosis, and the variable temperature reaction are noteworthy.

TEXT-FIG. 3. Effect on the blood count and temperature of the intratracheal inoculation of *B. Pfeifferi*. Third rabbit passage. The leucocytic rise, the polymorphonucleosis, and the variable temperature reaction are noteworthy.

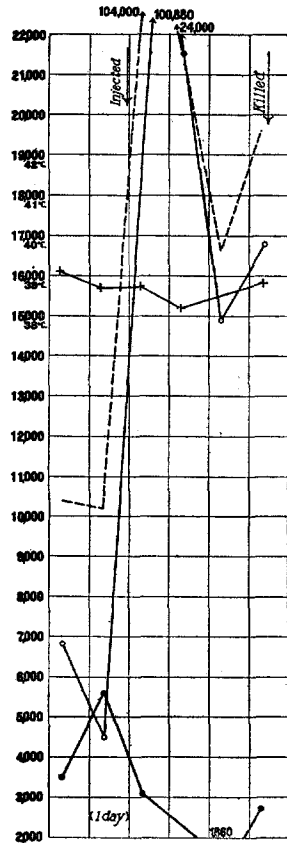
In other words, neither *B. Pfeifferi* nor its toxic constituents when injected intratracheally into the lungs of rabbits induced lung or blood changes similar to those described for the influenzal agent, or set up a pneumonic consolidation, or led directly to the death of the animals. As a rule, a polymorphonuclear leucocytosis without distinctive lung lesions was produced.

B. bronchisepticus.—Small doses (one-fiftieth of the 18 hour growth on a standard agar slant) induced after several days a slowly developing polymorphonucleosis and leucocytosis with multiple abscesses and necrosis in the lungs; large doses (ten times as much) caused acute death.

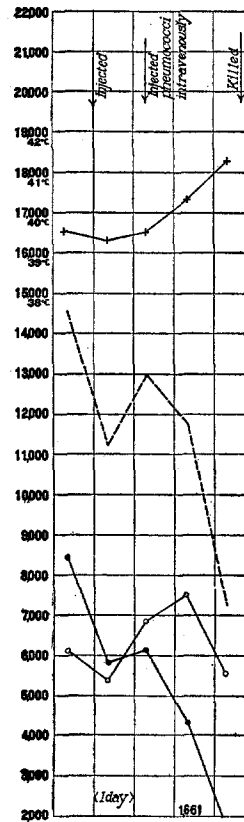
Experimental Reproduction of Concurrent Infections

We now turned to the experiments on concurrent infection with the influenzal agent and ordinary bacteria. To review the main points reached or at issue we may state that pneumococci, *Bacillus Pfeifferi* or its poison, and *Bacillus bronchisepticus*, in small doses, produce only transient effects when injected intratracheally in rabbits, and these effects differ essentially from the equally transient effects induced by the influenzal agent. It was next proposed to ascertain whether lungs already damaged by the influenzal agent would react differently to the bacterial inoculations. The method employed was to introduce the influenzal agent into the lungs and then to inject the bacteria either into the lungs or into the circulation. The latter route was adopted as a severer trial of the concomitant action, even though it may not be the route taken in the ordinary secondary infections of the lungs in man.

Influenzal Agent Plus Pneumococci.—May 31, 1919. A rabbit was injected intratracheally with 2.5 cc. of a suspension of glycerolated lung tissue corresponding to the fourteenth rabbit passage of the nasopharyngeal secretions from Patient 17, a case of influenza. June 1. Conjunctivitis; loss of weight (40 gm.); leucopenia (decrease of 2,075 cells); mononuclear depression (decrease of 612 cells). June 2. Injected intravenously with 5 million Pneumococcus Type IV, rabbit strain. June 3. Temperature 39.5°C.; total leucocytes increased 6,350 cells; mononuclears increased 4,904 cells. June 4. Temperature 40.5°C.; total leucocytes increased 2,875; mononuclears increased 54 cells. Died. The lungs showed consolidation of the right lower lobe, and hemorrhage, edema, and emphysema of other parts. From the lung and spleen Pneumococcus Type IV was isolated in culture.



TEXT-FIG. 4.



TEXT-FIG. 5.

TEXT-FIG. 4. Inoculation intratracheally of the poison of *B. Pfeifferi* as prepared by Parker's method. The marked polymorphonucleosis is shown.

TEXT-FIG. 5. Inoculation of a rabbit intratracheally with the influenza agent followed in 24 hours by an intravenous injection of pneumococci. The depression in the leucocytic as well as in the mononuclear counts, and the high temperature are noteworthy. This animal was moribund when killed, and showed lobar consolidation, which yielded pneumococci, besides the typical lesions of the influenza agent.

A second rabbit (Text-fig. 5) gave an identical result.

In order to control this experiment a third rabbit was injected intratracheally with 3 cc. of a suspension of normal rabbit lung tissue and then injected intravenously in the same manner as the preceding rabbit with 5 million pneumococci. Except for a transient leucocytosis 24 hours after the first injection, no effects were noted. The animal was killed 2 days after the injection of pneumococci. The lungs and other organs appeared normal.⁸

Influenzal Agent Plus Streptococcus viridans or B. Pfeifferi.—Experiments modelled precisely on those described for the pneumococcus were carried out with *B. Pfeifferi* and *Streptococcus viridans*. In each instance 24 hours after the intratracheal injection of the influenzal agent, a standard agar slant growth of *Streptococcus viridans* or one-half of such a growth of *B. Pfeifferi* suspended in saline solution was injected intravenously. Preceding the intravenous injections the characteristic blood changes due to the agent were present. The animals injected with the streptococcus usually succumbed on the 3rd day and showed a massive consolidation of the lungs with fibrinous pleuritis. Cultures from the lung tissue yielded an abundant growth of *Streptococcus viridans*. A control rabbit given normal lung tissue suspension intratracheally and the streptococci intravenously was killed after 3 days, and at autopsy failed to show lung changes. No growth of the streptococcus was obtained from the lung tissue.⁹

The combined influenzal agent and *B. Pfeifferi* injections set up a bronchopneumonic consolidation of one or more pulmonary lobes, and a hemorrhagic edema of the remainder of the lung. The cultures yielded abundant *B. Pfeifferi*. A control rabbit injected intratracheally with normal lung suspension and intravenously with *B. Pfeifferi* showed a transient polymorphonuclear leucocytosis. The lungs were not visibly altered.

These severe tests showed the conditions under which concurrent infections arose experimentally in the rabbit. A method was next employed in rabbits which imitated more closely the manner of development of secondary infections in influenza in man.

⁸ Larger or fatal doses of virulent pneumococci injected intravenously resulted in a generalized pneumococcal septicemia in rabbits inoculated intratracheally with normal rabbit tissue; all organs, including the lungs, were congested and yielded the pneumococcus on culture. The rabbits inoculated intratracheally with the influenzal agent, however, in similar procedures showed definite localization of the bacterial infection in the lungs—fibrinous and exudative pleuritis, and consolidation—besides the involvement of other organs.

⁹ In the case of *Streptococcus viridans* an experiment was also made in which these bacteria were injected intravenously 4 hours before the intratracheal inoculation of the influenzal agent. The results were similar.

Influenzal Agent Injected Intratracheally with Pneumococcus Type IV, Streptococcus viridans, or B. Pfeifferi.—A series of rabbits was injected intratracheally with the influenzal agent together with small non-pathogenic doses of Pneumococcus Type IV, *Streptococcus viridans*, or *B. Pfeifferi*. These experiments, properly controlled, showed that effects were produced similar to those obtained when these bacteria were injected intravenously; namely, more or less extensive consolidation, either lobar or bronchial, and from the affected lungs the same kind of microorganisms which were inoculated were recovered in pure culture, while the remainder of the lungs showed the hemorrhagic edema with emphysema characteristic of the influenzal agent (Figs. 1 and 2).

The experiments just given have an important bearing on the subject of this study since they show that the intratracheal injection of the influenzal agent in rabbits exerts an influence on the pulmonary structures of these animals of a nature to encourage the invasion of the lung and the subsequent multiplication there, with lethal outcome, of such bacteria as the pneumococcus, streptococcus, and *Bacillus Pfeifferi*, which otherwise, in the doses employed, are without marked effect. The control experiments show that the injection of normal rabbit lung exerts no such predisposing influence. While the experiments are perhaps not an exact reproduction of the conditions occurring in man in secondary pneumonia following influenza they bear directly on these conditions.

CONCLUSIONS.

1. Concurrent infections in the experiments described may be regarded as of accidental nature and are not causally related to the typical effects induced in rabbits by a material wholly free from ordinary bacteria.

2. The influenzal agent exerts an effect on the pulmonary tissue which encourages the invasion of the lung and subsequent multiplication there of ordinary bacteria, such as the pneumococcus, streptococcus, and *Bacillus Pfeifferi*.

3. A similarity is believed to exist between the conditions under which concurrent infections arose in the inoculated rabbits and those which seem to favor the occurrence of concurrent infections during epidemic influenza in man. In no instance did death occur in the rabbits as a result of the uncomplicated effects of the influenzal

agent alone. When death occurred in any of the inoculated animals concurrent infection of the lungs by ordinary bacteria was present. The microorganisms most commonly met with under these conditions were Pneumococcus Type IV and atypical Type II, streptococci, and hemoglobinophilic bacilli. Other kinds were encountered less often.

EXPLANATION OF PLATE 35.

FIG. 1. The influenzal agent with small numbers of Pneumococcus Type IV was inoculated intratracheally in a rabbit. The right lung shows the typical effects of the influenzal agent; it is voluminous, emphysematous, edematous, and hemorrhagic. The left lung shows massive lobar consolidation (red hepatization) and yielded on culture a profuse growth of the pneumococci. Natural size.

FIG. 2. The influenzal agent with *B. Pfeifferi*, both ordinarily without effect by themselves, was inoculated intratracheally in a rabbit. The left lung shows the typical effects of the influenzal agent; it is voluminous, emphysematous, edematous, and hemorrhagic. The right lung shows patchy consolidation (bronchopneumonia) and yielded on culture *B. Pfeifferi*. Natural size.



FIG. 1.

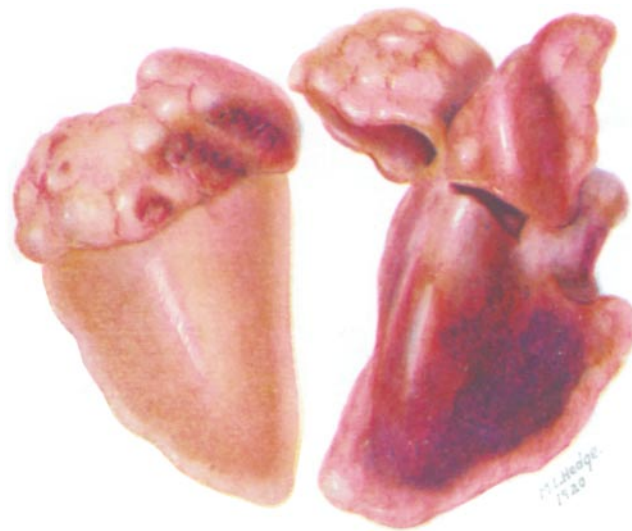


FIG. 2.