THE INFLUENZA BACILLUS IN INFLAMMATIONS OF
THE RESPIRATORY TRACT IN INFANTS.*

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The object of the following study is twofold: to determine the
presence of influenza-like bacilli in the throat and bronchial
mucus of children with and without pulmonary inflammations,
and to arrive at a differentiation of these bacilli by means of
cultural characters and agglutination reactions.

The material was chosen with a view to including as many
different pulmonary conditions as possible. A series of five
normal infants, whose history showed no cough since birth,
was first examined with negative results. Cases of coryza,
bronchitis, broncho-pneumonia, lobar pneumonia, empyæma,
rachitis, gastro-intestinal diseases, typhoid fever, diphtheria,
tonsillitis, measles, general sepsis following mastoiditis, rheuma-
tism, and cerebro-spinal meningitis, with and without pneumonia,
were next studied. The cases of coryza, bronchitis, and pneu-
monia were examined within from twenty-four to forty-eight
hours after admission, whether the onset had been such as to
suggest influenza or not. Only those cases were diagnosed "in-
fluenza" which showed the influenza bacillus in the bronchial
or nasal secretions. I am indebted to Dr. L. Emmet Holt for
the opportunity of studying twenty-two cases of measles at the
Foundling Asylum. Of the cases which came to autopsy at the
Babies' and Foundling Hospitals blood-agar plates were made
from the lungs irrespective of the cause of death.

The method employed was as follows: a sterile swab was
rubbed over the posterior pharangeal wall, care being taken to
avoid the tongue and the roof of the mouth. In most instances

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Medical Research.
this operation causes cough and the raising of some mucus from the bronchi, to which the swab attaches itself. Whenever the mucus was obtained in sufficient quantity, it was washed according to the method of Koch and Kitasato and then spread on blood-agar plates. A similar method was used by Jündell.\(^1\) Rabbit blood was used and mixed with the melted agar at 50° C. When only a very little secretion was obtained, this was spread at once upon the blood-agar plates. The throat cultures were always taken two or two and a half hours after a meal. The plates were examined after seventeen to twenty hours incubation. If no colonies of influenza-like bacilli were found, they were again incubated and re-examined the next day. Only those plates in which the colonies were definitely characteristic and proven by impression preparations to be made up of bacilli having the size, shape, and staining reactions of Bacillus influenzae, were regarded positive. In the majority of instances subcultures on blood-agar plates served to isolate these bacilli in pure culture, but in some I failed to obtain pure cultivations because of the presence of other organisms, chiefly cocci, in predominant numbers.

<table>
<thead>
<tr>
<th>CULTURES MADE DURING LIFE.</th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
<th>Total No. of Infants Examined.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16</td>
<td>37</td>
<td>53</td>
</tr>
<tr>
<td>Broncho-pneumonia</td>
<td>1</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Lobar-pneumonia</td>
<td>16</td>
<td>37</td>
<td>53</td>
</tr>
<tr>
<td>Normal baby</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Empyema</td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Coryza</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Tonsillitis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>General sepsis after mastoiditis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cerebro-sp. meningitis</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Rheumatism</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rachitis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gastro-intestinal diseases</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Typhoid fever</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Measles</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>6</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>
CULTURES MADE AT AUTOFSY.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broncho-pneumonia</td>
<td>3</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>With Pneum.</td>
<td>Without Pneum.</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>5</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Measles</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Enterico-colitis</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>General (umbilical) sepsis</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Prematurity (atelectasis)</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Marasmus (bronchitis)</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

The ages of the children varied from three days to four years, as follows:

- Under 3 months old: 18 cases
- Between 3 and 6 months old: 40 cases
- 6-12 months: 64 cases
- 1-2 years: 80 cases
- 2-3 years: 18 cases
- Over 3 years old: 8 cases

Total: 229 cases

The positive cases occurred during the months as follows:

- October: 30 cases examined, 10 positive, equal 33.3%
- November: 21 cases, 2 positive, 9.5%
- December: 19 cases, 10 positive, 52.6%
- January: 11 cases, 7 positive, 63.6%
- February: 46 cases, 12 positive, 26.1%
- March: 32 cases, 11 positive, 34.3%
- April: 48 cases, 9 positive, 18.7%
- May: 14 cases, 1 positive, 7.1%
- June: 1 case

The table gives a list of the cases studied, with the findings. Under “positive” are recorded the cases yielding influenza-like bacilli; under “negative” are recorded the cases from which the bacilli were not obtained.

Several varieties of organisms grew on the plates, although in many cases (pneumonia, empyema, tuberculosis) the colonies of influenza bacilli outnumbered the others. The bacteria which were present were: pneumococcus, streptococcus, Staphylococcus albus and aureus, Micrococcus catarrhalis, Diplococcus intracellularis (in a case of cerebro-spinal meningitis on the third day), diphtheria bacillus, pseudo-diphtheria bacillus, an un-
identified bacillus which was Gram negative and grew well on all culture media, and finally hemophilous bacilli resembling Bacillus influenza. The morphology of the last-named organisms was invariably in agreement with the descriptions of Bacillus influenza as given by Pfeiffer. The bacilli were sometimes polar-staining, occasionally forming threads, Gram negative, and growing only upon culture media containing haemoglobin. When inoculated upon serum-agar, with or without glucose, no surface growth took place, but bacilli could be found in the condensation water, in which they remained alive and transplantable to blood-agar for three days. Sub-cultures from the serum-agar tubes upon fresh tubes of the same medium were unsuccessful.

It would seem from the work of Ghon and v. Preyss that the influenza bacillus grows only in the presence of haemoglobin. The amount of haemoglobin required is, however, very small, as was shown in the course of Pfeiffer's work, in which the first generation was obtained on serum-agar. Pfeiffer explained the apparent discrepancy by assuming that the sputum used for inoculation contained mere traces of haemoglobin. Ghon and v. Preyss pointed out that an amount of haemoglobin too small to be demonstrated with the spectroscope, without the addition of hydrazin, is sufficient to insure the growth of influenza bacilli. Fichtner's cultivations on sputum-agar have not been repeated by others. A small bacillus resembling the influenza bacillus but differing from it by reason of its ability to grow on serum-agar has been obtained by Jochmann and Krause from cases of pertussis. The Koch-Weeks bacillus, which in some respects resembles influenza bacillus morphologically, is easily differentiated in cultures from the latter micro-organism.

In the course of this study several strains of the hemophilous bacillus obtained in cultures were compared. They came from the throat of a nurse (adult) who presented clinical symptoms of uncomplicated influenza; from the throat of an influenzal-pneumonia patient during life; from empyema pus; from the lung at autopsy of a case of tuberculosis with pneumonia; from the throat of a patient with measles; from the pus in a case of purulent conjunctivitis; from pertussis sputum.

In smears from the pus or sputum it was impossible to distinguish the several strains by differences in shape and size; but after several generations of growth they varied greatly in size of individual organisms and in the degree to which they formed short threads. Colonies as large as a pinhead, as described by Pfeiffer, were seen in all the strains; inoculations in
large amounts on perfectly fresh, moist blood-agar always brought about a return to the small, characteristic colonies. The very large, opaque colonies which Klieneberger described for the hemophilous bacillus isolated by him from the gall bladder in a case of empyema were not present among our strains, nor did any of our bacilli resemble in size Proteus vulgaris, as did the one noted by Klieneberger, who placed the bacillus isolated by him in the group of pseudo-influenza bacilli.

In a study of the agglutination reactions with the blood of all but two patients negative results were obtained in dilutions higher than 1:10. Cases of pertussis are not included in this series. The blood of the adult case of influenza reacted with the homologous bacillus in a dilution of 1:10 to 20 on the eighth day, and the blood from a case of intermittent influenza (see below) gave a reaction of 1:1 to 40 during the second and third attacks.

Rabbits were immunized with the strains indicated as "pneumonia," "tuberculosis," "adult influenza," and "eye," until their serum agglutinated the homologous bacillus in dilutions of 1 to 400. The other strains were then agglutinated by such sera in dilutions of 1:300 or 1:350, showing that the agglutinations were practically common to the group. Absorption experiments confirmed this conclusion. The following table gives the results of some agglutination tests.

Sera of rabbits immunized to influenzal-pneumonia culture:

<table>
<thead>
<tr>
<th>Dilution</th>
<th>1:10</th>
<th>1:20</th>
<th>1:100</th>
<th>1:200</th>
<th>1:400</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Pneumonia&quot; culture</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>&quot;Tuberculosis&quot;</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>&quot;Adult influenza&quot;</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>&quot;Autopsy pneumonia&quot;</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>&quot;Eye&quot;</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>&quot;Pertussis&quot;</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

In repeating my experiments of last year I found that these animals developed agglutinins much more slowly than the old ones had done, and the differentiation of the "pertussis" bacillus from the bacillus of "adult" influenza and "infantile pneumonia" was not so sharply marked as in the previous series. The agglutinins were common to the entire group, the "pertussis" strain, however, showing more frequent and greater variation in
agglutination than any of the other strains. The human blood reactions (excluding pertussis) were inactive to all strains, with the exception of the two cases mentioned above, in which the agglutination was too low for purposes of differentiation.

One bacillus which was isolated from a case of pertussis and measles-pneumonia gave reactions as high as those obtained with the “adult influenza,” “eye,” and “tuberculosis” strains. Whether this difference is to be explained on the ground that there was in this case a possible double infection with “pneumonia” and “pertussis” strains I am unable to say.

The absence of agglutination reactions with the serum from cases of acute pneumonia and bronchitis is to be explained probably by the fact that the agglutinins develop slowly. Rabbits require many inoculations before the agglutinins begin to appear. The blood of all the acute cases of disease was negative, the one positive reaction appearing in the case of intermittent influenza of longer standing. The positive agglutination reaction in pertussis is to be explained by the duration of the infection; and in these cases the reaction does not appear until the end of the second week of the cough.

My study on two occasions of the throats of five normal infants gave me no influenza-like bacilli whatever. Kretz found influenza bacilli in the throats of patients several months after an attack of influenza. He denominates these persons “influenza-carriers.” Rosenthal found Pfeiffer’s bacillus in the larynx and trachea in about one of six healthy persons, from which he deduced the saprophytic nature of Bacillus influenzæ. No adequate histories are given of the persons studied by him. Wassermann believed that whenever influenza bacilli are found influenza exists, a view which cannot be upheld, unless perhaps in the form given by Ortner, who holds that there cannot be influenza without the presence of influenza bacilli; although the bacilli may occur in the absence of the disease. Davis studied the throats of twenty normal persons and found small numbers of the bacilli in two of them. They had been free from any throat disease for three months, at least, before examination, and remained well for several weeks afterwards. These cases also suffer, in interpretation, from the absence of history of possible contact with persons suffering from influenza.

The cases of broncho-pneumonia and bronchitis had been admitted for these diseases; the stage of onset, therefore, had been passed outside the hospital. The children in whom the influenza bacillus was found were distinguished by showing higher
temperature, greater prostration, more illusive pulmonary signs, and longer duration of illness than the children suffering from broncho-pneumonia uncomplicated by influenza. In the cases of pneumonia and bronchitis which developed in the hospital the bacilli were found on the second day of the disease, before marked pulmonary signs had appeared.

In the three cases of empyema in which the pus contained the influenza bacillus, this organism had been obtained from the bronchial mucus before the operation.

Of the cases of tuberculosis twenty-four were studied, with eight positive results. Six of these gave physical signs of pneumonia. But among the sixteen negative cases, twelve gave similar signs. Eight of these cases came to autopsy: six with and two without positive findings. The question arose as to whether the presence of the bacillus influenced the clinical course of the disease. Of the positive cases five showed pulmonary consolidation, and one had acute miliary tubercles only. The last child showed as the oldest tubercular lesion a small cheesy nodule with a calcareous center in the left pulmonary apex. It seems not improbable that the influenzal infection lighted up this comparatively obsolete lesion.

Ortner observed twelve persons whose sputum contained both tubercle and influenza bacilli, and he remarks that the tuberculosis alone was sufficiently extensive to have caused death. In addition he reports a case of latent tuberculosis which became active and proved rapidly fatal after infection with influenza bacilli.

Ortner and Finkler describe "clinically intermittent influenza." The former means an acute, the latter a chronic influenzal infection of the bronchi. I encountered one such case: B. A., 15 months old, was a rachitic child with a severe anemia (hemoglobin 20%); the throat was negative for influenza bacilli. Having been discharged improved, she returned in about four weeks with cough, temperature 103°F, and fine rales over both bases. On the day of admission (Dec. 28) the sputum showed influenza bacilli in almost pure culture. On January 6, the lungs were clear. On January 27, the temperature rose to 103°F, an area of tubular breathing was found, and influenza bacilli were found in large numbers in the throat. On February 3, she was improved. On February 10, a throat culture was negative for influenza. On February 14, the fever and pulmonary signs returned, and cultures were again positive. For the first time the blood gave a positive reaction (1:30) with influenza bacilli. The child had three distinct attacks of acute influenza within three months, two with physical signs of consolidation of the lungs.

Pfeiffer called attention to the persistence of influenza bacilli in the lungs.
Influenza Bacillus in the Respiratory Tracts

of patients, but thought this especially true when the lungs are the place of diminished resistance before an attack of influenza, as in tuberculosis with cavity formation. Bronchiectasis is in this category, as is chronic bronchitis. Ortner 11 states that influenzal bronchitis may exist for months and may run an afebrile course. Lord 14 has also reported cases of long standing, and Boggs 13 observed the bronchiectatic cases. Davis 12 found influenza-like bacilli in the sputum of pertussis patients six months after the attack, and I have been able to cultivate them four months after the onset of the disease.

The influenza bacillus has been found in the nasal secretion in cases of measles by Liebscher 14 in eleven of fifty-seven persons examined during life; and in the lungs at autopsy three times. While 36 per cent. of these influenzal infected cases were fatal, the death rate among non-complicated cases was only 22 per cent. Süsswein 17 found the influenza bacillus in the nose in ten of twenty-one cases of measles and three times in the lungs at autopsy. Jehle 15 found the bacillus in the lungs in eighteen of twenty-three autopsies, and in the tonsils only once. The three writers just quoted consider that this double infection in measles influences unfavorably the clinical course. Jochmann, 19 on the other hand, finding the influenza bacillus in five out of ten autopsies in cases of measles complicated with broncho-pneumonia, believed that its presence has no such effect. Neither Davis 12 nor Klieneberger 6 expressed an opinion on this point. Albrecht and v. Preys 20 cultivated the organism from the lungs of an infant who died of pneumonia three weeks after an attack of measles.

Of the twenty-seven cases of measles which I studied nine gave cultures of influenza bacilli. The number of cases is too small to base any conclusions upon, but it may be remarked that of these nine cases, four terminated fatally, while of the eighteen negative cases seven died.

The presence of the influenza bacillus in the respiratory tract of patients suffering from measles in Europe and the United States at different seasons can hardly be considered as accidental or coincidental. As Ortner 11 has shown, influenza has never entirely disappeared from the continent of Europe since the great pandemic of 1889–90, but has become endemic (at least in the neighborhood of Vienna, from which city he writes), cases occurring every winter and spring. The same reasoning applies to New York City. The catarrhal conditions of the mucous membranes of the eyes and respiratory tract in the early stage of measles must make them favorable situations for the development of the influenza bacillus, which has, at all times, a predilection for these mucous membranes. And since the bacillus is probably always present in cities, in virtue of persistence of cases of chronic influenzal bronchitis and bronchiectasis, and
infection of the throats of persons recovered from influenza, the occurrence of influenza bacilli in a large percentage of cases of measles does not seem extraordinary. The frequent broncho-
pneumonic complication of these cases due often to the influenza bacillus speaks against the view of its saprophytic nature.

In twelve cases of purulent conjunctivitis which I studied I found bacilli belonging to the influenza group. Of these, three were children dying of cerebro-spinal meningitis in which throat cultures proved negative. From these cases a terminal ward infection of the eyes cannot be excluded. Four of the cases yielded the bacilli from the throat, one from the nose and throat, and one (throat culture negative) from the lungs at autopsy. The remaining three cases recovered and yielded negative throat cultures.

That the conjunctiva may possibly be the primary seat of infection with the influenza bacillus was stated by Zur Nedden, who found the eye alone involved in five of thirteen persons suffering with conjunctivitis due apparently to the presence of influenza bacilli. A. Knapp found this bacillus in the pus of a severe case of pseudo-membranous conjunctivitis in an infant six weeks old. No other symptoms of influenza existed, but the disease prevailed in New York at the time. Knapp states that there is an influenzal conjunctivitis without other manifestations of influenza. Wynekoop reports a case of very severe pseudo-membranous conjunctivitis from which the influenza bacillus was isolated in almost pure culture, there being mild general symptoms present.

One of the cases in my series is similar to the case reported by Wynekoop. It occurred in a fifteen-months-old child suffering from a pseudo-membranous inflammation of both eyes with much purulent discharge, from which the influenza bacillus was grown in pure culture. It was present also, with other bacteria in the nose. After several days a cough developed; the temperature ranged between 99.6° and 101.4° F., and influenza bacilli were found in the throat. Recovery took place in one week.

In my experience the influenza bacillus was not found in the throats of healthy children nor in the throats of children in whom lesions of the respiratory tract did not exist. When the bacilli occurred in the throat the clinical course of the disease which they complicated was, apparently, influenced by them. This fact seems to be especially true of pulmonary tuberculosis which runs a severer course when complicated by influenzal bacillus infection.

In interpreting the occurrence of influenzal bacilli in the throats of healthy persons, full histories of their previous
Health and association are necessary. The influenza bacillus persists for long periods after recovery from influenza, and the relapses of the disease may well be ascribed to this condition. Besides which, healthy individuals, themselves insusceptible, may well be the carriers of the bacilli to other and susceptible persons. Hence my experience is opposed, rather, to the view that the influenza bacillus is a harmless saprophyte.

I should also like to point out that the term "pseudo-influenza" bacillus should probably be discarded. The close similarity of cultural characteristics of all the influenza bacilli has been emphasized by Neisser, and my experience with the agglutination reactions leads me to regard all the strains as belonging to one family. The greatest diversity in regard to cultures and agglutination is shown by the influenza-like bacillus obtained from cases of pertussis; but even here I am not convinced that a separate and distinct species can be maintained.

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13. Finkler.—Quoted by Orter, loc. cit.