The Relationship of the Pneumococcus to Acute Infections of the Upper Respiratory Tract in Man

THE GRAM-NEGATIVE COCCI IN "COLDS" AND INFLUENZA

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BY

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THE RELATIONSHIP OF THE PNEUMOCOCCUS TO ACUTE INFECTIONS OF THE UPPER RESPIRATORY TRACT IN MAN*

INFLUENZA STUDIES VI

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In the present study, two general groups of acute respiratory infection have been investigated. The first group included a number of simple acute respiratory infections of varied clinical type, occurring in an urban population, and broadly classified as "common colds." The second was a recurrent epidemic of influenza occurring in the early winter months of 1920 and characterized by the same general clinical picture which was manifested in the great wave of influenza pandemic in 1918. The purpose of the work was to determine, first, the frequency with which the pneumococcus could be demonstrated in cases of influenza and in waves of common colds, and then to compare the extent of its incidence in these conditions with its occurrence in the upper respiratory tract of normal persons.

In the event that the pneumococcus was present to a greater extent in the pathologic respiratory tract than in the normal, it was essential to determine whether those pneumococci represented a single like strain in a given group of cases, or whether they were heterologous in nature and representative of unrelated varieties of that organism. Likewise, the pneumococcus isolated from the site of the primary lesion in common colds and influenza possesses a number of possibilities in its relationship to the infection. It may be present there normally as a part of the normal bacterial flora of that region and be involved in no way with the infection. It may, although normally present, be playing an accessory rôle in the inflammation. Again, it may be present as a secondary invader to the bacterium or virus causing the original infection. Lastly, it may be the true etiologic agent involved in the inflammation.

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* This is one of a series of studies carried out in connection with the Influenza Commission established and financially aided by the Metropolitan Life Insurance Company of New York. Part of the expense of these studies has been met by a grant from the University of Chicago.
With these various possibilities in mind, it was hoped by the general procedure outlined to acquire a certain amount of information bearing on the rôle of the pneumococcus in the etiology of "common colds" and influenza.

**BIOLOGIC DIFFERENCES AMONG PNEUMOCOCCI**

The study of the pneumococci isolated from patients with common colds and influenza and from normal persons, and the search for a particular organism which might be habitually concerned in the etiology of a given infection, has been largely facilitated by the advance, within the last few years, of our knowledge of the biology of the pneumococcus. It has been demonstrated that there exists within the species, certain well differentiated groups or types distinct one from the other.

Eyre and Washbourn,¹ in testing a serum made by Pané,² an Italian worker, for antibodies which would protect white mice against lethal doses of certain stock strains of pneumococci, found that in only 2 of 5 cases would this serum protect against his strains of cocci. Protection was not afforded against the other 3 in any appreciable dilution. This was the first indication of a biologic difference in strains of pneumococci.

Some years later Park and Williams⁴ showed that the organism described originally by Schottmüller⁵ as Streptococcus mucosus was really a pneumococcus, judged by its biologic properties, and should be classified as such, although differing morphologically from other varieties of pneumococci. Added evidence tending to include these organisms among the pneumococci was furnished by Collins⁶ through specific agglutination and agglutinin absorption tests. She furthermore contributed data tending to show that pneumococci in general could be separated into various groups by means of their agglutinative properties. Added observations by Hanes⁷ show decisively that these organisms are pneumococci, and that there is distinct cross agglutination among various members of the group. Neufeld and Händel⁸ demonstrated that there were well established varieties of pneumococci which, though resembling each other morphologically, nevertheless showed distinct immunologic differences. The work of Neufeld and Händel was followed by a more detailed study by Cole⁹ and by Dochez and Gillespie,¹⁰ the latter workers dividing pneumococci into 4 types or groups. These groups they designated as types 1, 2, 3 and 4. Types 1 and 2 differed from each other only slightly in morphology, but immunologically they were entirely distinct. These immunologic differences they demonstrated by animal protection and by agglutination tests. Group 3 included those organisms designated as Pneumococcus mucosus and were distinct from other pneumococci morphologically and serologically, although agreeing among themselves as a group. Group 4 was a heterologous division and included all cultures which did not fall into one of the other 3 groups. They resemble the organisms of types 1 and 2 morphologically, but do not possess common agglu-

⁶ Ibid., 1914, 19, p. 38.
⁸ Arch. Int. Med., 1914, 14, p. 56.
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tinative properties either with the other groups or among themselves. This
classification has been substantiated by subsequent investigations, those of
Lister, Mathers, Clough, Hartman and Lacy, Richardson, and Armstrong among others.

Later work by Avery on the classification of the group, showed that there
were certain strains of pneumococci which reacted atypically when agglutinated
with type 2 serum in that there occurred a partial or delayed reaction. Closer
study led to a separation of these atypical type 2 strains into 2 well-defined
serologic subgroups, type 2a and 2b with a heterologous subgroup type 2x.
Avery's experiments were extended by Stillman, and study of a larger num-
ber of such strains proved that atypical type 2 organisms could be subdivided
into at least 12 different subgroups. In fact, it would seem that there is really
evidenced within this group a repetition of what has occurred in the whole
series. Type 2 has a tendency to variation inherited from the original coccus,
with one of the subgroups type 2m still showing tendencies to subdivide.

Attempts to show some agreement among the heterologous type 4 strains
failed to demonstrate distinct division into subgroups. Fourteen strains studied
by Dochez and Gillespie seem generally to represent distinct varieties (Cole).
Olmstead, however, reports, from a study of 213 group 4 strains, that they
can be divided into about 12 subgroups by cross agglutination experiments.

METHODS OF STUDY

The method of collecting material for subsequent examinations is of
importance, and preliminary experiments were made to determine the
most favorable procedure. The collection of sputum was out of the
question because bronchial secretions are not raised in the majority of
cases. All material was obtained therefore, by swabbing the mucous
membrane with sterile cotton swabs placed on iron wire, after the
type of the Mathers swab.

All patients were examined clinically by another member of the
staff, Dr. W. B. Sharp, who was working at the same time on the
epidemiologic and clinical phases of acute upper respiratory infections.
I am indebted to Dr. Sharp for all clinical data contained in the present
report. Depending on the clinical diagnosis, certain areas of mucous
membrane were thoroughly stroked with the swabs.

The first 25 cases included 13 of acute rhinitis, 2 of acute follicular
tonsillitis, 5 of acute pharyngitis, and 5 cases which presented involve-

10 South African Institute for Medical Research (Publications), Dec. 22, 1913.
17 Ibid., 1919, 29, p. 251.
ment of the bronchi together with acute inflammation of the upper respiratory tract. In order to obtain data on the most favorable site from which to obtain material, separate swabs were taken from the nose, the nasopharynx and the pharynx. Individual examinations were made for the pneumococcus from each of the swabs so taken, according to the methods outlined later. Briefly, it was found that cultures from the nasopharynx gave the greatest percentage of pneumococci irrespective of diagnosis and indicated that region to be a primary seat of localization for the organism in acute respiratory infections of this class. In cases of rhinitis, throat cultures were found to be of little value, but the nasal cultures on two occasions gave positive results when material was taken well back toward the choanae, while nasopharynx cultures proved negative. In inflammations of the pharynx, larynx and tonsils, nasal cultures proved of no value, indeed cultures from the region of the pharynx were positive for the pneumococcus in only 1 of 6 cases. As a result of these preliminary examinations the following routine procedure was adopted: In cases of rhinitis, the middle fossa and floor of the nostril was swabbed, together with a second swabbing of the nasopharynx. In cases of tonsillitis, pharyngitis, laryngitis and influenza, swabblings were made from the posterior pharynx and from the nasopharynx. Normal persons were swabbed in all three areas, nasopharynx, nostril, and the pharynx. Particular care was taken in all swabblings to prevent salivary contamination. Separate determinations were not made. The two swabs were pooled, and a single determination made from the combined material by injection into mice.

The methods for the demonstration of pneumococcus in the material collected as described offered certain technical difficulties. Early in the studies, the swab was placed in a tube of sterile, warmed Ringer solution, and the mucous secretions brought into suspension by allowing the swab to stand in the warm fluid for about 2 hours with frequent agitation. One c.c. of the suspension was then injected intraperitoneally into a white mouse. The method first advanced by Blake 20 for the determination of the presence and type of pneumococcus in sputum was then followed, essentially as recommended by him. The results were not satisfactory in that few pneumococci were obtained, and the mortality of the mice was very low. Two swabs were taken from a given area and treated in the same manner as had been the single swab;  

it was hoped thereby to obtain a heavier inoculum. No appreciable improvement could be observed. Finally, the method was modified to include a preliminary enrichment of the material on the swabs, followed by subsequent inoculation of this enriched fluid into mice. This modified method has given excellent results and is essentially as follows:

The two swabs taken from the most promising regions, as determined by clinical examination, the nasopharynx and either the pharynx or nasal cavity, were placed in 5% sheep dextrose blood broth, made according to the method which Avery 21 used in his substitute method for mouse technic in the typing of pneumococci from sputum. Our technic differed only that sheep blood was substituted for rabbit blood. The broth was then incubated for a period of from 8 to 12 hours at 37 C., with agitation of the tube from time to time to insure proper distribution and full benefit from the added blood. This method gives a preliminary enrichment of the pneumococci contained on the original swabs. Stained smears from such broth cultures will ordinarily show a preponderance of pneumococci over the accompanying staphylococci, within the time of incubation used. Following the preliminary enrichment, 1 cc of the broth culture, as free as possible from red blood corpuscles, was injected intraperitoneally into white mice. In cases in which death of the animal did not follow within 24 hours, a peritoneal puncture was made. Smears were made from the fluid withdrawn and, satisfactory growth being evidenced, the animal was killed.

The peritoneal cavity of the mouse was washed according to the method of Blake 20 and the pneumococci typed by agglutination and precipitin tests in the usual manner. Cultures on blood-agar plates were made at necropsy, from peritoneal fluid and from heart blood. The gram-positive, lance-shaped, encapsulated diplococci were later isolated in pure culture, and confirmatory agglutination and bile solubility tests were made with the pure broth culture for final determination of the type of pneumococcus. All agglutination tests were given 2 hours' incubation at 37 C., and a preliminary reading made. They were then placed in the icebox over night and final readings made in the morning. Bile solubility tests, as the final criterion of a pneumococcus, were made by adding one part of ox bile, first filtered and then sterilized by steaming for 3 succeeding days, to 3 parts of a

broth culture of the organism. In all instances final diagnosis of organism and type were based on these reactions in pure broth culture.

The experience of many workers has seemed to indicate that passage of suspected pneumococcus material through a white mouse furnishes probably the most exact technic for its isolation. The animal tissues furnish a favorable environment for the growth of the organism, and in addition the blood stream of the white mouse exercises a more or less specific filtering action on the pneumococcus. Nevertheless, it was felt that careful examination of plate cultures from the swabs might yield even better results. Consequently, in a series of 56 cases, duplicate plate examinations and mouse determinations were carried out. The medium used for the plates was a 5% sheep blood veal infusion agar, reaction Ph 7.8.

The plates were inoculated with the swabs and then streaked out in sunburst fashion. After 24 hours’ incubation at 37 C., they were examined under the dissecting microscope and 5 green-producing colonies, selected at random from different portions of the plate but showing minute differences in colony structure, were picked to blood agar slants. These were later obtained in pure broth culture and subjected to bile solubility tests. The mouse technic was as previously outlined.

Of the 56 cases subjected to duplicate examinations, 13 proved positive by the mouse method while only 4 gave positive cultures of pneumococci by the plate method. In only one instance was a pneumococcus found by the plate method and not obtained by the mouse technic. The results show that the technic of enrichment and subsequent mouse inoculation is best suited to the isolation of pneumococci present in these conditions. Following these preliminary experiments in the autumn of 1919, the mouse method was employed exclusively in further studies.

THE INCIDENCE OF THE PNEUMOCOCUS IN NORMAL PERSONS

The incidence of the various types of pneumococci in the pneumonias has been determined for a large number of cases, not only by the original workers, Dochez and Gillespie, but by numerous other investigators previously cited; likewise, data are at hand bearing on the evidence of the organism in normal persons.
Pneumococci and Respiratory Infections

Pneumococci are frequently found in saliva. Early studies by Frost, Divine and Reineking 22 demonstrated pneumococci in the saliva of 18 of 50 different normal persons examined, or 36%, although their criteria for determination of the coccus were probably not exact in the light of our present-day knowledge. Study of the sputa from 70 normal persons by Park and Williams 8 demonstrated pneumococci in 51 instances, although the same criticism might apply to their work, in that bile solubility tests were not employed. Stillman,23 in a comprehensive study conducted some years later, and covering about 297 cases of normal persons, demonstrated the organism in 39% of his cases. The incidence of types was also determined. He found that types 4 and 3 were the most common groups of pneumococcus observed in normal saliva, with atypical type 2 organisms showing a relatively high incidence. The fixed types, types 1 and 2, were found in only a single instance. It would seem that the incidence of the pneumococcus in saliva is relatively high, averaging probably 40%, with certain definite types predominating.

A distinct necessity in the study of an organism concerned in acute respiratory infections is the determination of a mean for that organism in normal persons in sites most subject to acute inflammation. Certain data are available concerning the incidence of the pneumococcus in the nasopharynx and pharynx of such normal persons. Sailer, Hall, Wilson and McCoy 24 studied 700 men in a military command. These men were free from respiratory infection, but had been in more or less contact with prevailing pneumonia. Swabs were taken from the nasopharynx, spread on blood-agar plates, typical colonies picked, and broth cultures subjected to the usual tests of bile solubility and agglutination for type. One hundred and eleven of these cases yielded cultures of the pneumococcus, or 16% of the total. Of the positive findings 5.4% were of type 1, 13.52% were type 2, type 3 constituted 4.5% while 76.58% were found to fall into the type 4 group. A later study of a civilian population by Meyer 25 showed 21 of a series of 100 normal subjects to be carriers of the pneumococcus, or 21%. Of the 21 cultures isolated, none were classified as members of the fixed types 1 and 2. One type 2a strain was identified, while 3 proved to be of type 3. The remaining 17 belonged to the heterologous type 4. Fifty samples

of sputum from various persons among a dispensary population were studied by Sydenstricher and Sutton and gave similar results, in that 38% were positive for the pneumococcus, with type 4 predominating.

A further study of pneumococcus incidence among normal persons by the same methods employed for the study of respiratory infections was felt to be essential, and preliminary to any studies of the nose and throat under pathologic conditions. Briefly, 46 cultures have been made from a group of students in the university who had not at the time been knowingly exposed to colds, or who had themselves suffered from colds within recent date. All were subjected to a careful clinical examination before cultures were taken to rule out possible inflammation of the respiratory mucous membrane. From the cultures derived from 10 of these persons, pneumococci were obtained, 21.7%. The majority of these were type 4, nine of the cultures falling into this group. The other culture was of atypical type 2 variety. Strains representative of the fixed types were not found, nor were strains of type 3 isolated. The swabbed material, even after preliminary enrichment, was in general nonpathogenic, inoculations of 1 cc of the blood broth culture from swabs, producing death of the mice within 24 hours in only 2 instances in the entire series. The incidence of the pneumococcus, then, in the normal respiratory tract was found to be somewhat over 21%, with most of the organisms type 4, and possessing a low grade of virulence.

THE PNEUMOCOCCUS IN COMMON COLDS

The results obtained by a considerable number of investigators previously cited, has led to definite knowledge of the incidence of the different types of pneumococci in pneumonia; likewise, the incidence in normal persons is rather well understood. Few studies, however, have been made on the prevalence of the types of pneumococci in simple acute respiratory conditions. Valentine has probably made the most extensive study of the pneumococcus in common colds. Her investigations were directed toward a determination of the pneumococcus in these simple infections as a possible source of contagion for lobar pneumonia. Cases in her series numbered 65, and pneumococci were recovered from 43 of them. The types were represented by two strains of type 1, two of type 2, four of type 3, while 35 were placed in the heterologous type 4.

Among the pneumonia cases studied by Clough were included a few cases of acute and chronic bronchitis in which pneumococci were found. Other than these two reports, no information bearing on the frequency of pneumococci in the simpler respiratory conditions could be found in the literature.

In studying the cases of common colds in our series, no attempt was made to select certain types of infection. All cases among a student population were examined as they were reported from time to time. Consequently, they vary considerably in the clinical type of infection. A special effort was made, however, to have cases available early in the onset of the inflammation, and the majority were observed within the first 24 hours of the cold, rarely after a period of more than 48 hours following the onset of symptoms. A total of 77 colds are included in the present series. Briefly, 27 of them showed the pneumococcus when cultivated by the methods outlined. The incidence of the different types of pneumococci is indicated in table 1.

| TABLE 1 |
| The Pneumococcus in Common Colds, Together with the Incidence of Types of Pneumococci |

<table>
<thead>
<tr>
<th></th>
<th>Total Cases</th>
<th>Pneumococcus Present</th>
<th>Percentage Showing Pneumococcus</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>46</td>
<td>10</td>
<td>21.7</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Colds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute rhinitis</td>
<td>47</td>
<td>17</td>
<td>36.2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Acute pharyngitis</td>
<td>9</td>
<td>1</td>
<td>11.1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Acute rhinitis and bronchitis</td>
<td>11</td>
<td>5</td>
<td>45.5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Acute pharyngitis and bronchitis</td>
<td>4</td>
<td>2</td>
<td>50.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tonsillitis</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Incidence of pneumococci in all colds: 77 cases, 27.49\%

Percentage of incidence of types in positive cases of colds: 3.7\% Type 1, 7.4\% Type 2, 14.8\% Type 3, 14.8\% Type 4, 59.2\% Type 4

The results presented in table 1 indicate that the pneumococcus is present in a considerably higher percentage of cases of colds than in the series of normal persons examined under the same conditions. No particular variation in the degree of incidence among the various types of inflammation is evidenced, except that in general it may be observed that pneumococci are found more commonly in those cases which show an involvement of the bronchi, together with an inflammation of the nose or pharynx.
By far the most common group of coccius found in normal persons was the pneumococcus 4. This was also the case with pathologic throats, but in these representatives of all of the types were isolated, even the fixed types 1 and 2 being observed.

No attempt was made to determine the exact number of pneumococci in relation to members of the gram-negative, streptococcus and Pfeiffer bacillus groups. Rough plate readings were made, however, and in general, green-producing colonies constituted from one third to three fourths of the total colonies developing on blood-agar plates, inoculated directly with the swabs from the nasopharynx.

A STUDY OF THE PNEUMOCOCCI INVOLVED IN EPIDEMIC SORE THROAT

The cases of acute respiratory infection which have been previously described constitute sporadic cases developing among a typical urban population. In the course of this work, a restricted epidemic of sore throat occurred among the children attending the School of Education of the University of Chicago. The clinical picture of these cases was out of the ordinary. This fact, together with the evident communicability of the infection, attracted attention to the epidemic which apparently was localized in this particular institution. It has been studied intensively by Sharp, Norton and the writer, and a complete report will be given in a later paper. Certain data which are of importance in a general consideration of the relation of the pneumococcus to upper respiratory infections, will be given here briefly.

A series of nine cases was selected from among those first encountered in the epidemic, and examinations were made for a period of approximately three months at weekly or bimonthly intervals. An additional examination was made after the lapse of 4 months, when a recurrence of the pathologic condition developed, following summer vacation.

A type 4 pneumococcus was isolated consistently from all 9 patients throughout the course of the infection and for periods varying from 2 weeks to at least six weeks after all clinical symptoms had disappeared. In several recurrent cases developing after 5 months, in the same institution, pneumococcus 4 was again isolated in most of the cases. Serologic identity of all of these type 4 pneumococci has been proved. This work is described later in the course of this paper.

A number of normal throats, 13 in all, were examined in the course of the study for the presence of pneumococci in order that a control
might be obtained on cases of the infection. The results obtained from
the examination of 2 such series, selected at random from approximately
the same age group and the same school population, showed that the
particular organism observed in the pathologic throats was uniformly
absent in the normal throats. Pneumococci were demonstrated in
several instances, among them four type 4 and two type 2a pneumococci.
These type 4 organisms were, however, proved to be serologically
distinct from those involved in the epidemic, with a single exception.
The latter instance might well be explained on the basis of the individ-
ual representing a normal carrier condition.

THE PNEUMOCOCCUS IN INFLUENZA

In the course of our studies on common colds, there occurred in the
early months of 1920 a recurrence of the 1918 pandemic of influenza.
Work on colds was suspended for the time being, and investigations
were confined to a similar study of the incidence of the pneumococcus in
influenza. On account of the brief period during which cases were
available, only a limited number could be studied. Three separate
series were investigated in order that the bacteriology of like conditions
in different localities might furnish some basis for comparison and
possibly permit a generalization on bacteriologic conditions in this type
of infection.

The attention of various investigators had been called to the prevalence of
pneumococci in influenza in the epidemic of 1890, and greater or less stress
has been laid on its importance in this condition ever since. Even before
Pfeiffer 28 called attention to the bacillus which was held for so many years
to be the direct etiologic agent in influenza, the pneumococcus had been studied
in its relation to the disease. Marmorek 29 was among the first to encounter
the pneumococcus in influenza. Weichselbaum 30 in more searching studies,
found the pneumococcus to be among the most frequent invaders. Further
evidence concerning the presence of the organism in influenza and its sequelae
was furnished by Kirchner, 31 Babes, 32 and Levy. 33 At this time the organism
was apparently felt to have no special significance in the direct etiology of
the condition. The general inference of all of these workers was essentially
that the virus of influenza, whatever it was, merely prepared the way for the
entrance of the pneumococcus as a secondary invader.

In the most recent pandemic, that of 1918, a great number of workers found
the pneumococcus in cases distributed throughout the country. In certain

28 Deutsch. med. Wehnschr., 1892, 18, p. 28.
30 Ibid., p. 104.
districts it seemed to be the chief organism involved. Lamb and Brannin\(^4\) found it to be among the more important organisms in influenza at Camp Cody. The extended study by Hirsch and McKinney\(^5\) at Camp Grant indicated the pneumococcus to be the chief organism involved in cases occurring at that post during the height of the epidemic. Later studies\(^6\) on postepidemic cases, however, demonstrated a high incidence of hemolytic streptococcus infections, less frequently pneumococcus. In general, one may say that all workers recognized the pneumococcus as constituting one of the three or four more important groups of bacteria concerned in the infection. In some localities, it was the dominant species.

The group of cases included in the present report is derived from three different sources. A number of cases developed among the same general student population at the University of Chicago from which we had been drawing our material for the study of common colds. A group of cases occurring at the Great Lakes Naval Training Station constituted our second series, while the third series was obtained from soldiers stationed at Camp Grant, Rockford, Ill. The results of the examinations from these three series of influenza cases are contained in table 2.

\[
\text{TABLE 2} \\
\text{THE PNEUMOCOCCUS IN INFLUENZA}
\]

<table>
<thead>
<tr>
<th></th>
<th>Total Cases</th>
<th>Pneumococcus Present</th>
<th>Percentage Showing Pneumococcus</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 2(a)</th>
<th>Type 3</th>
<th>Type 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Lakes</td>
<td>17</td>
<td>6</td>
<td>36</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Camp Grant</td>
<td>12</td>
<td>4</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>University of Chicago</td>
<td>8</td>
<td>4</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Incidence of pneumococci in all cases.</td>
<td>37</td>
<td>14</td>
<td>38</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Percentage of incidence of type pneumococci in positive cases.</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>7</td>
<td>21</td>
<td>7</td>
<td>14</td>
<td>50</td>
</tr>
</tbody>
</table>

Although the total number of cases is too small to warrant definite conclusions, the results indicate in a degree that the presence of the fixed types is considerably more common in influenzal conditions than in common colds. It even approaches somewhat the type incidence usually encountered in lobar pneumonias. Pneumococci were not present in influenza cases much more commonly than in common colds. Data on cases of cold showed an incidence of 34%, in influenza, 38%. The organism was present about twice as frequently in influenza as it was in the series of normal persons studied.

\(^6\) Ibid., 1919, 25, p. 393.
AN ATTEMPT TO DETERMINE THE SPECIFIC RELATIONSHIP
OF THE PNEUMOCOCCUS TO COMMON COLDs

Experimental data have been presented which furnish evidence of
the presence of the pneumococcus in common colds and influenza, and
furthermore indicate that in normal persons these organisms can be
found in about 21% or more of cases. It seemed desirable to obtain
information bearing on certain fundamental questions. Is the partic-
ular pneumococcus which is found in such an acute respiratory infection
merely present, more or less accidentally, as a normal inhabitant of the
tract at the time of the developing infection? If so, does it evidence
any activity, any symbiotic relationship to the infecting bacterium or
virus during the course of the infection? If not, does it gain entrance
into the inflamed area coincidently with developing symptoms, previous
to that time, or somewhat later in the course of the infection? Does it
disappear soon after the subsidence of clinical symptoms, or may it
persist for appreciable lengths of time, constituting a carrier condition,
permanent or temporary? With these questions in mind, a group of 10
normal persons was selected, and each one was subjected to daily
routine cultures over a period of about two and one-half months. Each
case was watched closely for the onset of any symptoms which would
indicate an oncoming cold. Detailed daily studies of each culture
were not made after the initial culture, except when colds developed.
But from time to time, at intervals of perhaps two weeks, such examina-
tions were made in order to maintain a general idea of the constitution
of the flora. The plates were critically examined each day, however,
and a percentage estimate made of the different types of colonies
which developed.

During the course of these experiments, 5 of the 10 subjects in the
series developed one or more colds, the other five remaining normal
throughout the period of observation. Among the persons who main-
tained a normal condition of the upper respiratory tract, different
conditions were observed as regards the pneumococcus.

Subject A-4 gave cultures of the pneumococcus of the type 4 variety
on the initial examination, and this organism persisted in subsequent
cultures over a period of 2 months, green producing cocci being the
predominating organisms in the flora continuously. It then disappeared
and further cultures proved negative.

Early plate readings of A-5 showed the absence of green producing
cocci in the flora. Fifteen days after the first examination, green
colonies became dominant on the plates. Cultural tests showed that they included pneumococcus 4. They continued in the throat until 2 months after the beginning of the study of the case, when B. mucosus capsulatus became dominant, and indeed crowded out the green-producing colonies. Cultures taken after this time yielded no pneumococci.

The group of green-producing coci was the predominating type of organism in the cultures from A-7 throughout the course of the study. Pneumococcus 4 was identified.

Subject A-8 was characterized by the presence, largely, of only 2 of the general groups of respiratory micro-organisms during the period of examination, namely, green-producing cocci and organisms of the Micrococcus catarrhalis group. No pneumococci were isolated at any time.

The flora of A-10 was found to be subject to considerable variation. Early in the course of the experiments, the Mirococcus catarrhalis group predominated, with the greens numerically next in order. Two months after the first examination, the greens increased markedly in numbers and pneumococci belonging to type 4 were isolated. This condition was only transitory, however, for soon the Pfeiffer bacillus dominated all plates with the pneumococcus no longer capable of being demonstrated in culture.

It would seem, then, that the flora of the normal person, at least during the winter months, is subject to distinct variations in constitution from time to time. One type of organism may dominate in a given respiratory tract for varying lengths of time, to be eventually supplanted by another. In 4 of the 5 cases studied, the pneumococcus was found at one time or another. It persisted in a given throat for as long as 2 months or more, and constituted a distinct carrier condition. Again, it was present in demonstrable numbers for a few days only, with subsequent replacement by other species of bacteria. In these 5 cases the pneumococcus came and went, or persisted indefinitely, without the development of clinical symptoms.

Somewhat more uniform conditions are evidenced among the 5 patients who developed colds in the course of the experiments. Green producing cocci were present in the upper respiratory tract of A-1 from the time of the original culturing, and extending over two months time. Pneumococci were, however, not present, the green-producing cocci being Streptococcus viridans. The average incidence varied from 20
to 50% per cent of the total flora, as evidenced by plate cultures. Occasionally green colonies dominated the plates to the practical exclusion of all other forms. Two weeks after initiating cultures, an acute pharyngitis developed. Pneumococci were not demonstrated in the course of the cold. About 2 months after the original culturing, a second acute pharyngitis was manifest. The first day on which symptoms were present no pneumococci were present in cultures, and the percentage of greens had not noticeably increased. The second day's culturing gave the same bacterial picture. On the third day, however, pneumococcus 4 was isolated. It was absent in cultures taken one week later, although slight symptoms of the cold persisted.

Two colds developed in the course of the study of A-2. Symptoms of a beginning rhinitis were present on the second day after study of the case had been initiated. Pneumococci could not be isolated throughout the course of the cold, which lasted 8 days. Three weeks later a second rhinitis developed, and this time a pneumococcus 4 was obtained from cultures taken on the first day that symptoms of the inflammation were present. The organism persisted in cultures taken during the course of the cold but disappeared with the subsidence of the infection.

A-3 likewise developed an acute rhinitis the second day after the beginning of the investigation. Eight complete examinations were made during the course of the infection, but the pneumococcus could not be demonstrated in culture. Six weeks after the first infection, a second rhinitis was observed. A culture was not obtained until the second day after the beginning of the cold, but at that time the pneumococcus 4 was found in large numbers. During the four days on which symptoms were manifest, the organism was demonstrated each day, and it continued in cultures for 10 days thereafter. Following that time it remained negative during the 3 successive observations.

Green-producing cocci were uniformly in the minority in cultures taken from A-6, oftentimes being absent. The gram-negative cocci and staphylococci were the predominating organisms in the flora. Three weeks after the first culture, an acute rhinitis was diagnosed. The Pfeiffer bacillus seemed to be the organism chiefly involved. Pneumococci were not isolated from any of the cultures taken during the study of this case.

The fifth subject who developed a cold while under observation was A-9. Green-producing cocci were consistently the predominating type in
cultures, usually constituting about 90% of the colonies developing on blood-agar plates, but no pneumococci could be determined. Three weeks after the initiation of the study, a slight rhinitis followed. A type 4 pneumococcus was cultivated on the first day that symptoms appeared, but it disappeared from cultures soon, and subsequent cultures were negative for the organism.

Analysis of the last 5 cases brings out certain facts. Certainly the pneumococcus was not involved in the etiology of all of the colds developing in the series. The first colds of subjects A-2 and A-3 could not be connected in any way with an etiology involving that organism; nor could the cold which was observed in A-6. The second colds occurring in both A-2 and A-3, as well as the rhinitis which was observed in A-9, may have been of pneumococcal origin. In these 3 cases of rhinitis, the pneumococcus was present in cultures coincidently with the onset of symptoms. As regards the second cold of A-1, the question is problematical, but most likely the pneumococcus isolated in that instance can best be interpreted as a secondary invader. One cannot, however, definitely ascribe a pneumococcal origin to even these 3 cases, for there is no direct knowledge of the period of incubation of such infections. The presence of the pneumococcus at the time of infection may have meant merely that under the stimulus of the exciting agent of the cold, pneumococci normally in the throat, although present in such small numbers that they could not be demonstrated, rapidly began to multiply, and possibly acquired as well a distinct pathogenicity. Thus one cannot determine whether the presence of the organism indicates direct etiology or symbiotic relationship to the infection.

From the study of this series of cases, then, one is led to conclude that intermittently normal persons may develop either a temporary or even chronic carrier condition for Fränkel's pneumococcus, without the organism causing any symptoms of pharyngeal or nasal inflammation. A certain percentage of colds may be due to the invasion of the respiratory tract by the pneumococcus since, in the cases cited, pneumococci have been demonstrated not to be present in the normal condition of the nose and throat. Furthermore, evidence of a beginning inflammation was present coincidently with their determination in culture. In other circumstances it appears that the organism invades the inflamed area secondarily to some other organism which has primarily incited the infection.
THE EXTENT OF THE CARRIER CONDITION FOR THE PNEUMOCOCCUS

During the course of the study of the "A" series of cases previously described, it was observed that a given pneumococcus was carried in the throats of persons a relatively short time following convalescence from these simple types of respiratory involvement, acute rhinitis and pharyngitis. Ten days was the longest time observed in the cases studied. These few cases would indicate that there is little danger of spread of contagion from the convalescent.

In the cases of epidemic tonsillitis and pharyngitis described previously, the School of Education Series, the convalescents maintained the carrier condition for a much longer time. The majority of the 9 patients in this series revealed the pneumococcus in examinations following the infection for at least 6 weeks, most of them 2 months or longer, while in 3 patients it persisted for at least 7 months. It must be remembered, however, that this was an unusual type of respiratory involvement and, moreover, the particular pneumococcus 4 was distinct from any other subgroup of pneumococcus 4 isolated in the course of our studies on colds.

Attention was directed to the possibility of a certain percentage of normal persons constituting a population of permanent pneumococcus carriers. A series of normal persons was studied in order to ascertain whether the carrier state as observed in normal persons was transitory in type or was as a rule long continued. Six subjects were examined during the spring months, April and May, while a second group of 5 were studied intensively during the early winter months, Nov., Dec., and Jan. Four cases of the Spring series, when examined at weekly intervals, did not at any time give evidence of pneumococci of any type. Two cases did present pneumococci at some time during the experiments. One subject, C623, was positive at the first examination, and continued to give cultures of type 4 pneumococcus for three weeks, after which the cultures were negative. The second subject, C492, proved negative for pneumococcus at the first examination but one week later cultures were positive. Cultures were still positive about 7 weeks later when conditions prevented further study.

Of the series of 5 normal cases studied during the early winter months, 4 at some time or other picked up the organism, although all were initially negative. A temporary carrier condition developed,
respectively, for periods of 2 months, 2 months, 1 week, and 3 weeks, in the 4 cases.

The limited number of cases studied forbids definite conclusions. Indications are that protracted carrier condition of the pneumococcus is rare but that temporary carriers develop with considerable frequency, probably more commonly in winter months than during the spring and summer. Based on the cases studied, the average time of the carrier state is about one month.

SEROLOGIC STUDY OF TYPE 4 CULTURES TAKEN FROM PERSONS WITH COLDs, FROM NORMAL PERSONS AND, FROM PERSONS WITH INFLUENZA

Pneumococcus 4 has been by far the most common pneumococcus observed in our study of acute respiratory infections. Previous studies cited have shown a decided variation in the relation of any one strain of the type 4 pneumococcus to another, and indeed the group is recognized as a collection of heterologous strains having little serologic relationship. So common, however, was its occurrence in our cases of various clinical type, that if any particular organism, for example the pneumococcus, was to be given any weight in a determination of a common etiology, some specific relationship necessarily had to be demonstrated between these various strains. In other words, was a single variety of pneumococcus 4 involved, or did the various strains differ in biologic properties?

Fifteen strains were selected from type 4 pneumococci isolated from various types of acute respiratory involvement and from normal persons. These 15 cultures, representing type 4 pneumococci present in normal persons, in cases of influenza, and in various types of common colds, have been studied intensively.

Monovalent rabbit serums were prepared for these 15 strains by injecting the animals with cultures of pneumococci grown on blood-agar slants and washed off in salt solution. Killed cultures were used in the early injections and were given intravenously for 3 successive days, followed by a like period of rest. Later live cultures were employed. As a general rule, about 20 inoculations were required to obtain a relatively high agglutination titer for the coccus. All serums so prepared, agglutinated the homologous strain in a dilution of at least 1:600, some as high as 1:1,600. After a sufficiently high titer had been obtained, the rabbits were bled to death, and the serum preserved in
frozen ampoules. Serum so preserved will not show a decided drop in agglutinating power for some time. One serum, stored for 9 months, had lost only 30\% of its agglutinating value.

All 15 strains were tested by agglutination with each of the immune serums so prepared. The agglutinating titer for the homologous strain as well as cross agglutination with other strains, was determined for each serum. The tests were made by adding 1 c c of a young broth culture of pneumococci to an equal volume of diluted serum. Control tests included monovalent horse serums for types 1, 2 and 3, in dilutions of 1:40, 1:40 and 1:20, respectively, a normal rabbit serum control, in a final dilution of 1:25, and a saline control. The tests were incubated at 37 C. for 2 hours, preliminary readings made, and the tubes placed in the icebox over night. Final results were recorded from readings made the following morning.

**TABLE 3**

**CROSS AGGLUTINATION OF TYPE 4 PNEUMOCOCCI**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Source</th>
<th>Monovalent Rabbit Serums</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C423-4</td>
</tr>
<tr>
<td>C428-4</td>
<td>Normal</td>
<td>800</td>
</tr>
<tr>
<td>C492-7</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>E-24</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>E-27</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>G-4</td>
<td>Influenza</td>
<td>1000</td>
</tr>
<tr>
<td>G-6</td>
<td>Influenza</td>
<td>1000</td>
</tr>
<tr>
<td>O 027</td>
<td>Influenza</td>
<td></td>
</tr>
<tr>
<td>O 028</td>
<td>Influenza</td>
<td></td>
</tr>
<tr>
<td>O 230</td>
<td>Acute rhin-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>itis,........</td>
<td></td>
</tr>
<tr>
<td>C 201</td>
<td>Acute rhin-</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>itis,........</td>
<td></td>
</tr>
<tr>
<td>O 163</td>
<td>Acute rhin-</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>itis,........</td>
<td></td>
</tr>
<tr>
<td>C 054</td>
<td>Acute rhin-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>itis,........</td>
<td></td>
</tr>
<tr>
<td>C 230</td>
<td>Acute rhin-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>itis,........</td>
<td></td>
</tr>
<tr>
<td>O 235</td>
<td>Tonsillitis</td>
<td>25</td>
</tr>
<tr>
<td>O 114</td>
<td>Acute rhin-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>itis,........</td>
<td></td>
</tr>
</tbody>
</table>

* Figures represent highest agglutinating titer; — = absence of agglutination.

Control tests were all negative with the exception that strain C201 was agglutinated by type 1, 2 and 3 monovalent horse serums. The normal rabbit serum and saline controls for this strain were negative.

The cross agglutination experiments indicate common properties peculiar to certain of the organisms studied. Final group classification and identity of strains was proved by the use of the agglutinin absorption technic. Those serums which showed agglutination with strains
other than the specific organism used in its preparation, were absorbed by the cross agglutinating organism, and the absorbed serum then tested by agglutination for the persistence of agglutinins for the homologous pneumococcus.

The technic of agglutinin absorption was as follows, essentially that used by Avery \(^{16}\) in his study of atypical type 2 strains. The washed bacterial residue from 150 c c of an 18-24 hour old broth culture was added to the undiluted serum. Before adding the organisms, they were killed by heating to 55 C. for one-half hour. The mixtures were incubated at 37 C. for 2 hours with frequent agitation, and then were placed in the icebox over night. The serum was then freed of bacteria by centrifugation, and the supernatant fluid pipetted off. This absorbed serum was used for agglutination tests with the homologous organism for the presence or absence of agglutinins. The technic was controlled by the absorption of the serum by the homologous organism as well, with agglutination tests following, in order to assure that the amount of bacterial residue used was sufficient to remove all agglutinins.

By these methods it was determined that certain of these type 4 strains fell into restricted groups. Strains G4 and G6 from the Camp Grant epidemic of influenza and strain C201 from a case of rhinitis in the Chicago series proved to be allied. Contact between the three patients was unlikely with the exception of the 2 patients with influenza. Likewise, strains C163 and C235, isolated respectfully from cases of acute rhinitis and bronchitis, and from acute tonsillitis, were found to constitute a second small group. Strains C230 and C548 also possessed like serologic properties. No relationship could be established between C504, a rhinitis strain and C623-4, a normal strain, although cross agglutination had occurred. Agglutination had been due apparently to minor agglutinins. Strains C492-7, C623-4, E24, E27, C627, C628, C504 and C114, all differed in biologic properties.

Immunologic experiments on the predominating type of pneumococcus isolated in the course of this study show that there is little relationship between such strains encountered in various acute respiratory conditions. Of the 15 studied, 8 were entirely distinct one from the other. Grouping could be effected in 3 instances, one group including 3 of the strains studied, the others 2 each. It seems certain that there is little relationship between the type 4 pneumococci encountered in the acute infections of the upper respiratory tract. In the light of studies made on type 4 pneumococci occurring in lobar pneumonia, this was logically to be expected.
Distinctly different results have been obtained in a study of the group 4 strains obtained from those cases of epidemic tonsillitis and pharyngitis previously described, which occurred in the School of Education at the University. Common agglutinating properties were found to exist between all strains isolated from the original 9 cases studied, as well as from 2 others observed later. A monovalent rabbit serum prepared from strain E4-6 agglutinated all of the strains mentioned in the foregoing. Cross agglutination was not obtained with type 4 strains from other respiratory infections or from normal sources. Control tests with the fixed types were likewise negative. Serologic identity of all these strains was thus established.

In general, then, one may conclude that the pneumococci which are encountered in various types of respiratory involvement are unrelated. There is no common strain which is involved in the etiology of these infections, and no specific etiology may be claimed for the pneumococcus. In isolated instances, however, such as the epidemic at the School of Education, a common strain of pneumococcus may be involved in a given group of cases.

THE COMPARATIVE VIRULENCE OF PNEUMOCOCCI ISOLATED FROM NORMAL THROATS AND FROM THROATS AFFECTED BY ACUTE RESPIRATORY CONDITIONS

The observation was made during the study of material from various sources by the mouse method that a difference in degree of pathogenicity for these animals was apparent. Cultures from normal throats which were later proved to contain pneumococci had little effect and indeed rarely produced death of the animal within 2 or 3 days. Cultures from patients with cold differed. A few would cause death of the mice in 24 hours or thereabouts, but most of them had a decidedly lesser effect. During the study of influenza cases it was apparent that the organisms involved were much more virulent for mice.

Of course the original injections given the animals contained a variety of organisms besides the pneumococcus, and the variation in lethal effect therefore could not definitely ascribed to a difference of virulence among the pneumococci themselves. For this reason a comparative study of pure strains of pneumococci from various sources has been made. It is well known that the virulence of the pneumococcus varies greatly according to the length of time it has been carried in artificial culture. It gradually decreases in virulence. Likewise its
virulence for a given species can be enhanced by frequent passage through that animal. In order that results might be directly comparable, all of the tests for virulence have been made by using blood-agar cultures having the same history. These pneumococci, after removal from the throat, had undergone one mouse passage, had been cultivated on blood-agar plates and then picked to blood-agar slants. They were, then, in the second generation on artificial medium after a single mouse passage. By this method, conditions were exactly comparable and, furthermore, tests for virulence were made within the shortest possible time following removal of the organism from the throat.

In obtaining cultures for inoculation, care was taken to seed as nearly as possible the same extent of blood-agar medium. Growths were washed off in 5 cc of warm sterile broth, and appropriate dilutions made in broth so that the final inoculum was contained in 0.5 cc volume. Mice were inoculated at once intraperitoneally. The results obtained with cultures from normal persons, from patients with colds and from patients with influenza are given in table 4.

**TABLE 4**

**Comparative Virulence for Mice of Pneumococci Isolated from Various Sources**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Source</th>
<th>Type of Pneumococcus</th>
<th>Amount of 24-Hour Blood-Agar Slant</th>
</tr>
</thead>
<tbody>
<tr>
<td>G623-4</td>
<td>Normal</td>
<td>IV</td>
<td>0.01 0.001 0.0001 0.00001</td>
</tr>
<tr>
<td>C492-7</td>
<td>Normal</td>
<td>IV</td>
<td>S     S     S</td>
</tr>
<tr>
<td>E 22</td>
<td>Normal</td>
<td>IV</td>
<td>S     S     S</td>
</tr>
<tr>
<td>E 28</td>
<td>Normal</td>
<td>IV</td>
<td>S     S     S</td>
</tr>
<tr>
<td>G 4</td>
<td>Influenza</td>
<td>IV</td>
<td>D14 D15 D60 S</td>
</tr>
<tr>
<td>G 6</td>
<td>Influenza</td>
<td>IV</td>
<td>D14 D48 S S</td>
</tr>
<tr>
<td>C 504</td>
<td>Acute rhinitis</td>
<td>IV</td>
<td>D14 D48 S S</td>
</tr>
<tr>
<td>C 548</td>
<td>Acute rhinitis</td>
<td>IV</td>
<td>S     S     S</td>
</tr>
<tr>
<td>GL 10</td>
<td>Influenza</td>
<td>II</td>
<td>D14 D13 D14 D60</td>
</tr>
<tr>
<td>GL 12</td>
<td>Influenza</td>
<td>II</td>
<td>D12 D14 D14 D22</td>
</tr>
<tr>
<td>C 290</td>
<td>Acute rhinitis</td>
<td>IV</td>
<td>D48 S S S</td>
</tr>
<tr>
<td>C 201</td>
<td>Acute rhinitis</td>
<td>IV</td>
<td>D69 S S S</td>
</tr>
</tbody>
</table>
| C 163   | Acute rhinitis and bronchitis | IV | D12 D16 S S

* S = survived 72 hours; — = not tested; figures represent number of hours before death.

Pneumococci from normal throats uniformly failed to cause death of the mice within the period of observation, even with the largest doses used. Cultures derived from patients with influenza were considerably more virulent than those from normal persons, even in comparison with the same group of organism, type 4. Likewise cultures from persons with colds possessed a higher degree of virulence than type 4 cultures from normal throats.
Pneumococci and Respiratory Infections

Discussion

Pneumococci are observed in various simple inflammations of the upper respiratory mucous membrane, grouped together under the general term of "common colds," somewhat more frequently than in throats which do not show lesions, although the increased percentage incidence to the particular region affected, namely rhinitis, pharyngitis, tonsillitis to the particular region affected, namely rhinitis, pharyngitis, tonsillitis or combinations of these conditions with bronchial involvement, do not show any appreciable degree of difference in the frequency with which pneumococci are encountered. If any conclusions may be drawn from the limited number of cases studied, it would appear that the organism is somewhat more common in infections which include involvement of the bronchi.

In patients with influenza, pneumococci are frequently encountered, but again in numbers not much greater than in normal persons, and slightly increased over the incidence in common colds. The frequent occurrence of fixed types is chiefly of interest in comparison with the types of pneumococci found in common colds and in normal persons.

While the patient with the average common cold or influenza will only show the presence of the pneumococcus in somewhat greater frequency than the ratio of 1:3, nevertheless outbreaks of respiratory infection may occur in which this particular coccus is involved in practically all cases. Such an instance was demonstrated in our School of Education series.

By observing the bacterial changes evidenced in oncoming colds, it was found that in certain instances pneumococci appear in the throat practically at the same time that developing symptoms are observed. Since there is no exact knowledge of the period of incubation in common colds, one can merely conjecture as to the significance of the pneumococci present. If common colds present the relatively short incubation period which we know is characteristic of influenza, then it would seem that in some cases this organism may be the direct causative factor. In other instances, the presence of the pneumococcus in the inflamed area was demonstrated relatively late in the course of the infection. It would seem to be present in such cases as a secondary invader.

Serologic studies of pneumococci, involving type determinations of the organisms from pathologic throats and a more careful study of the predominating group, type 4, give evidence that no one variety of
pneumococcus is concerned in those cases in which the organism is present in one capacity or another.

Interesting information is furnished by the study of the relative virulence of pneumococci from various respiratory infections and from normal sources in respect to their relationship to disease. Our early individual case studies and the results obtained in our "A" series in which developing colds were observed both pointed to the conclusion that the pneumococcus certainly could not be involved in the etiology of all colds. The serologic study of various strains obtained from those cases in which pneumococci were found led to the definite conclusion that no common etiology can be proved, for the strains vary decidedly in biologic properties. What, then, is the province of the pneumococcus in these infections, or has it really no significance? Some light is cast on the problem by the virulence studies. Pneumococci from cold sources are surely more virulent for mice than are cultures derived from normal persons. In other epidemics, such as influenza, it shows a still more heightened virulence.

Granting the fact that the pneumococcus may only play a primary etiologic rôle in a small percentage of cases, as indicated by the studies of our "A" series, still it would seem that the pneumococcus may rather commonly be a secondary invader; or, if present normally in the throat in numbers too small for detection, may increase rapidly in numbers, and under the stimulus of the conditions generated by the inflammation, or a symbiosis with the infecting virus, acquire an increased virulence. It seems probable that in a considerable percentage of upper respiratory infections the pneumococcus is in reality pathogenic, and exercises a real influence in the course of the infection.

CONCLUSIONS

The average incidence of the pneumococcus in normal throats, in the present series of cases is about 21%. Cases of common colds showed pneumococci more commonly, 35%, and the same was true of influenza, 38%.

Type incidence of pneumococci in normal persons and in persons with colds was characterized by the infrequent occurrence of the fixed types of pneumococci. Fixed types are somewhat more frequent in influenza than in other acute infections of the upper respiratory tract.

No serologic relationship could in general be demonstrated between the pneumococci found in these infections. Types other than 4 were
observed. Type 4 strains in this series show a division into 8 different strains and 3 small groups. No common strain of pneumococcus was present in acute respiratory infections.

A particular group of cases occurring in a localized epidemic showed a uniform occurrence of the pneumococcus in practically all of the cases studied, a type 4 pneumococcus. The strains were proved to be serologically identical. It seems certain, then, that although the general statement can be made that pneumococci are not found in the majority of colds, and that when they do occur they are rarely related to each other, nevertheless instances occur in which a single type of pneumococcus is involved in all cases of a given group.

Comparative virulence tests showed that the pneumococci from colds and from influenza were more pathogenic than the strains from normal throats.
THE GRAM-NEGATIVE COCCI IN "COLDS" AND INFLUENZA*

INFLUENZA STUDIES VII

J. E. GORDON

From the Department of Hygiene and Bacteriology, University of Chicago

No affection is more common in temperate zones, probably, than that group of conditions, varying largely in clinical type and manifestations, which are classed together as "common colds." Relatively little investigation has, however, been directed toward the determination of their etiology. Their usual mild course and the absence of a fatal termination doubtless account for this fact. The tremendous economic loss caused by the incapacitation for work, from time to time, of a considerable proportion of the population, gives them an importance not always recognized. The reduced resistance following colds is likewise important in that it often predisposes to more severe infections, not alone confined to the respiratory tract. It would seem desirable to obtain more exact knowledge of the cause and mode of spread of common colds and influenza.

The varied flora of the upper respiratory tract, under both normal and pathologic conditions makes the problem of the bacteriologic investigation a most complicated one. Recent studies by Bloomfield\(^1\) have contributed materially to our knowledge of the bacteria which are normally present in the throat, and to the conditions which govern invasion by organisms not commonly present. Some circumstance other than the mere presence of a given bacterium seems to be necessary for the initiation of disease. Large amounts of bacterial growth, from cultures of various organisms, Sarcina lutea, Staphylococcus albus, B. coli\(^2\), B. influenzae of Pfeiffer,\(^3\) B. mucosus capsulatus of Friedländer\(^4\) and Streptococcus hemolyticus,\(^5\) were smeared on the tongue, pharynx, nasal septum and into the tonsillar crypts of persons free from unusual abnormalities of the upper air passages. The various organisms disappeared as a rule within 24 hours. In no case was any demonstrable lesion or general reaction set up. The mechanical flushing action of the secretions seems undoubtedly the most important element in the disposal of bacteria introduced into the

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* This is one of a series of studies carried out in connection with the Influenza Commission established and financially aided by the Metropolitan Life Insurance Company of New York. Part of the expense of these studies has been met by a grant from the University of Chicago.

\(^1\) Am. Rev. Tuberc., 1920, 4, p. 247.
mouth and nose. The saliva in many instances appears to be an unfavorable medium for bacterial growth. The outstanding fact seems to be that the "normal" intact mucous membranes of the upper air passages are not only impervious to the attack of certain pathogenic bacteria, but that these organisms also fail to colonize on such surfaces. Bloomfield tentatively concludes that there are certain bacteria which constitute a true mouth flora in the sense that they live and multiply and are almost constantly present in most people and persist from day to day over considerable periods of time. Another group of organisms may be occasionally recovered from the mouth, but as a rule they are present for only short periods of time. They seem to be bacteria which are essentially transients and fail to colonize or to survive on normal mucous membranes. Occasional individuals are encountered who "carry" over considerable periods of time organisms which do not belong to the normal true mouth flora.

The micro-organisms which have been studied in greatest detail in their relation to colds and to influenza are the pneumococcus, the varieties of streptococi, and the Pfeiffer bacillus. In addition to these three major groups, there are commonly present in infections of this class, a large group of cocci which are decolorized by the Gram method. Some of these are conceded to be saprophytic inhabitants of the normal nose and throat. Others are regarded as more or less foreign to the normal mucosa and ordinarily present only in pathologic processes.

Studies of the meningococcus during periods of epidemic meningitis have furnished a wealth of information concerning the gram-negative coccal flora of the nose and throat in that infection. The first extended investigation was conducted by von Lingelsheim who described various species of gram-negative cocci, including the meningococcus. Elser and Huntoon report at length on the gram-negative cocci observed in epidemic meningitis. Added observations have been contributed by Dunham. Certain general groups have been reported by all of these workers. The various species, then, which might be expected in such a study as the present one, would include rather rarely the meningococcus, more commonly M. catarrhalis and a large group of chromogenic cocci, and in addition certain organisms such as M. pharyngis siccus, Diplococcus crassus and diplococcus mucosus, of less frequent occurrence.

The study of this group of gram-negative cocci, particularly M. catarrhalis, in their relation to the etiology of common colds and influenza, forms the basis of the work here reported.

Two general groups of acute respiratory infection have been investigated. The first included a number of common colds of varied clinical type, occurring in an urban population. The second group was composed of cases from a recurrent epidemic of influenza, developing in the early winter months of 1920, and characterized by the same general clinical picture which was manifested in the pandemic of 1918.

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6 Ibid., 1920, 31, p. 118.
6 Klinisches Jahrbuch, 1906, 15, p. 373.
It was decided in the preliminary experiments, to determine whether or not there was any decided increased incidence of gram-negative cocci in respiratory infections, over the occurrence in the normal throat. Furthermore, was any particular kind of gram-negative coccus found more commonly in pathologic than in normal throats? By this general procedure it was hoped to acquire a certain amount of information bearing on the rôle of M. catarrhalis and the gram-negative group in common colds and influenza.

Mere demonstration of an increased incidence of a given organism in respiratory infections can, however, simply furnish a clue to its significance. Such an organism may have been present as a normal inhabitant of the mucous membrane at the time of the developing cold, and be involved in no way in the infection. It may, although normally there, be playing an accessory rôle in the inflammation. Again, it may be present as a secondary invader to the bacterium or virus primarily causing the infection. Finally, it may be the true etiologic agent. Experiments involving a determination of the progressive bacterial changes in oncoming colds can furnish valuable information on these points.

METHODS OF STUDYING THE BACTERIAL FLORA IN UPPER RESPIRATORY INFECTIONS

Two general sources of material may be taken as available in a study of this nature. Sputum is assuredly preferred in a study of the bacterial flora of the pneumonias, and might be considered as desirable in studies of the simpler infections of the respiratory tract which present bronchial involvement. Involvement of the bronchi is, however, the exception rather than the rule in the usual type of "common cold." Even when present, the sputum raised is at best scanty and irregular. Consequently, the study of the bacteriology of all upper respiratory infections has been confined to examination of material obtained by swabbing the area of infected mucous membrane.

It seemed logical to suppose that the infecting organism in a given inflammation, whether it be a gram-negative coccus or any organism, would be present in greatest numbers on the mucous membrane first affected. Physical findings were determined by another member of the staff, Dr. W. B. Sharp, to whom I am indebted for all clinical data contained in this report. Depending on the diagnosis made, certain areas were subsequently swabbed. Early in the course of the experiments it became evident that gram-negative cocci were of extremely
common occurrence in the nasopharynx. Later studies have confirmed this observation. Of the first 75 cases in which gram-negative cocci were found, 65 gave cultures from the nasopharynx. An exact record was kept of the region from which each strain of gram-negative coccus was isolated. Twenty-five cases showing gram-negative cocci, were swabbed from the nasopharynx and tonsillar regions. Nineteen of the nasopharynx cultures were positive while 20 from the posterior pharynx contained gram-negative cocci. These cultures were chiefly derived from cases of pharyngitis and tonsillitis, sometimes accompanied by bronchial involvement. Another series of cases were subjected to duplicate swabblings from the nose and nasopharynx. Of 50 cases in this group in which gram-negative cocci were demonstrated, 46 gave cultures from the nasopharynx against 30 from the nasal cultures.

The region of the nasopharynx is the chief site for localization of gram-negative cocci in the pathologic upper respiratory tract. Next to the nasopharynx, they are found with greatest frequency in the tonsillar region, with the anterior nares showing the lowest incidence. On the basis of this preliminary work, a routine procedure was adopted for study of all colds. Cases diagnosed as rhinitis were swabbed from the middle fossa and floor of the nostril and from the nasopharynx, while all other inflammations, those of pharynx, larynx, tonsils or combinations of these cases with bronchial infection were swabbed from the nasopharynx and from the tonsillar region. Patients with cases of influenza were likewise swabbed in the nasopharynx and pharynx. Ordinary wooden applicators with sterile cotton swabs were used for the nasal and tonsillar swabs, while the bent wire with cotton swab, as introduced by Mathers, was employed for obtaining nasopharyngeal material. Particular care was taken in making nasopharyngeal and tonsillar swabs to avoid salivary contamination.

Methods of Culture.—Three different mediums were employed at the beginning of the work, the vitamin-blood-pour-agar plates of Park and Williams, the oleate-hemoglobin-agar of Avery and a 5% sheep-blood agar, made from a veal infusion base, with 2% agar added, and the reaction adjusted to $P_{H}$ 7.8. The vitamine blood medium has been suggested as a general enriching medium and especially favorable to growth of the meningococcus. It seemed a highly
desirable medium for culture of all gram-negative cocci. The oleate-hemoglobin medium was developed late in the course of the recent pandemic of influenza, and has been found by numerous workers to be especially suited to the culture not alone of the Pfeiffer bacillus, but of all gram-negative organisms. Plain veal infusion blood agar has been so universally suited to cultivation of organisms from respiratory conditions that it was included as the third medium.

Careful records were kept of the results obtained from the use of each of the three mediums, during the early part of the work, that their comparative value might be determined. Not only was the incidence of the various kinds of gram-negative cocci determined, but a rough percentage estimate was made of the number of colonies developing, as compared with other groups of respiratory organisms. Of the first 75 cultures, which by one medium or another gave gram-negative cocci, it was found that growths on the oleate hemoglobin plates were positive in 66 instances. The veal infusion blood agar was almost as satisfactory, in that 55 of the cases gave positive cultures on this medium. The vitamine-blood-pour plates left much to be desired. Only 32 of 75 cases, proved by other methods to include these gram-negative organisms among their flora, were positive when cultures were made on this medium. Oleate-hemoglobin agar seems to be the medium best suited for growth of gram-negative organisms from the nose and throat, although plain blood agar is almost as satisfactory. On the basis of these results, the vitamine medium was dispensed with in later work, but cultures were still made on both blood agar and oleate-hemoglobin agar, as it was felt that the factor of using an increased number of plates would in itself tend to give higher percentage findings of the organism for which we were looking. At least 4 plates, and in the early work 6 plates, have been inoculated with material from each case studied. Swabs taken from 2 areas were rubbed over a small area on the surface of the plated medium. The inoculations were then streaked with a looped needle in “sunburst” fashion. Incubation followed at 37 C. for 24 hours. Special care was taken to maintain a high moisture content in the incubator, since study of meningococci has demonstrated the decided importance of this factor in the growth of gram-negative cocci.

The plates were examined under a dissecting microscope for the appearance of characteristic gram-negative-like colonies. These colonies were fished to blood agar slants made by the same formula as the
medium employed for blood-agar plates. An average of 10 colonies were fished to slants from the plates of each case, although no attempt was made to limit the number and oftentimes more were taken.

Colonies characteristic of the gram-negative cocci are readily distinguished on blood-agar medium from other types of respiratory organisms. One kind of gram-negative colony is typically that of the meningococcus. It presents a characteristic small, transparent bluish white colony which is regular in outline and of a watery consistency. The usual catarrhalis-like colony grows somewhat more luxuriantly than that of the meningococcus. Under the microscope the colony appears darker, more compact and has rather ragged margins. It has a granular appearance, especially toward the center of the colony, is semitransparent and causes no change of the medium. A third general type of colony characteristic of gram-negative cocci is one slightly smaller than the meningococcus-like colony and readily differentiated by its consistence. The colony is firm and adherent to the surface of the medium. So firm is the colony that it can readily be lifted with the needle and turned over bodily. Diphtheroid colonies are distinguished from the gram-negative-coccus colonies, with difficulty. Determination of morphology furnishes the only sure method. Staphylococci are readily differentiated by their larger size and distinct white appearance. Colonies of streptococci and pneumococci with their attendant change in appearance of the medium furnish no difficulty, nor do the colonies of the Pfeiffer bacillus.

Few colonies other than those of gram-negative organisms develop on the oleate-hemoglobin agar. This fact, together with the growth favoring qualities which it possesses, makes it a valuable medium. Occasional colonies of diphtheroids may be confused with those of gram-negative cocci. Staphylococci are commonly present, but are distinguishable by their larger size and white appearance.

After 24 hours' incubation the blood agar slant cultures picked from plates were definitely classified as belonging to the group of gram-negative cocci by morphologic examination. The Sterling ¹² modification of the gram-strain has been used throughout the work. The gentian violet stain was applied for 10 seconds, the iodine solution for 20 seconds, followed by alcohol. Ten per cent. aqueous saffranin solution, applied for 1 minute, has been used as a counter stain. With each new lot of stain prepared, control preparations were made, to

insure proper differentiating value. A culture of a known meningococcus was employed as a negative control, a culture of Staphylococcus aureus as a positive control.

Cultures distinguished on the basis of morphology and Gram reaction as members of the gram-negative coccus group were reserved for classification and further study.

Vedder's starch medium was found to be very well suited for carrying stock cultures of the various gram-negative cocci. Practically all strains encountered grew readily on this medium, especially after a few generations. A few were refractory and stock cultures in these instances were maintained on blood agar until growth could be initiated on starch agar. Freshly isolated cultures were transferred at weekly intervals for the first two or three generations, after which monthly transfers sufficed. Every third or fourth transfer of stock was put onto blood agar instead of starch agar, and after a week retransferred to starch agar. It is a matter of common experience that stock cultures of these organisms are maintained with difficulty, for occasionally a strain will die out, even after long artificial cultivation and for no apparent reason. By these methods, however, unusually good results have been obtained.

THE CLASSIFICATION OF GRAM-NEGATIVE COCCI OF THE NOSE AND THROAT

The essential purpose of this investigation has been a classification of the gram-negative cocci which occur in the normal and pathologic upper respiratory tract. Having effected a classification, the incidence of the different groups in various types of common colds and in influenza will be discussed, and an attempt made to determine their probable relation to the given infection.

Primary classification has been made on the basis of reaction toward various carbohydrates. General laboratory practice has favored the use of liquid carbohydrate mediums in the classification of bacteria by fermentation reactions. In our work, however, a solid medium has been employed, and has been found to be much more satisfactory and more easily controlled. Liquid medium is preferable in a study of organisms which give fermentation with production of gas, in that roughly quantitative measurements of the gas may be made. None of the gram-negative cocci heretofore described possess this property. The only other advantage possessed by a liquid medium is the fact that
the degree of acidity may be measured in a broth medium by a determination of the hydrogen-ion value. This may likewise be done within rough limits for a solid medium, by preparing samples of different pH value, and observing color values with a standard indicator. There seems then to be no decided disadvantage possessed by a solid medium in a study of this particular group, and its advantages are material. Many of the gram-negative cocci grow with difficulty in broth media. On solid medium the growth is much more satisfactory. Growth in broth can often only be determined by stains and morphological examination. On solid medium growth is readily determined. A large proportion of these organisms, furthermore, do not produce acid from any carbohydrate, and in liquid medium, especially if the broth be somewhat turbid, morphologic examination must frequently be made to insure conclusive readings. Probably the most distinct advantage possessed by an agar-base medium is the control furnished of purity of growth of the inoculated strain, and freedom from contamination during the technical procedure of transferring. Growth characteristics readily demonstrate contaminations on solid medium. Only examination of the morphology will serve definitely to control broth cultures. For these reasons, it was felt that use of medium involving an agar base would not only facilitate technical operations, but would also guard against erroneous results.

The carbohydrate mediums have been made according to the following formula: One pound of finely chopped lean veal is infused with 1 liter of distilled water for 24 hours on ice. At the end of this period the liquid portion is removed by use of a meat press and the albuminoid substances coagulated by bringing to the boiling point. The infusion is then filtered, and the total volume brought up to 1 liter. To this veal infusion 0.5% sodium chloride, 1% peptone and 2% agar are added and the substances brought into suspension. The reaction is adjusted to pH 7.8 and 1% Andrade solution added as an indicator. Sugar solutions previously prepared in distilled water were added to give a final concentration of 1%. The medium was then steamed for 3 successive days in the Arnold sterilizer. It was then slanted, care being taken to leave at least an inch of solid medium in the butt of the tube below the sloped surface. The medium was incubated for 24 hours at 37° C. for sterility. Each lot of medium was inoculated with a known fermenting organism to insure proper reaction.

Both stab and streak inoculations were made on the several carbohydrate mediums. It is well recognized that growth of certain of the gram-negative cocci is enhanced by a partial reduction in oxygen tension, and conditions best favoring growth were found as a rule to prevail at the butt of the tube, although surface growths were uniformly good. In some few instances growth could not be initiated, but it was found that by smearing the surface of the agar with a small amount of sterile, inactivated sheep serum that growth could always be obtained. This technic was employed for such strains.

Purity of growth in cultures was insured by making pour plates of all cultures just before they were tested for fermentation. Single colonies were fished from the plates to agar slants and examined for morphology. The medium was made in large amounts, and so far as possible strains were tested for acid production in considerable numbers at one time in order that comparable conditions of temperature, moisture and the same lot of medium might prevail.

Cultures were given an initial incubation of 24 hours at 37 C., and preliminary observations were recorded. Incubation for 7 days followed, when final readings were taken. Varied reactions have been obtained by making these double readings. Some organisms were found to be rapid acid formers, the reaction at the end of 7 days being no different from that taken at the end of the 24-hour period. The majority were slow acid producers, there being a negative reaction at 24 hours with a distinct positive reaction at the time of final observation. A third limited group produced a slight degree of acidity with certain of the sugars during the first 24 hours. Final readings at 7 days, however, showed a return to alkalinity.

The reactions to 8 sugars have been studied, namely, dextrose, levulose, and galactose of the monosaccharids, lactose, saccharose and maltose of the disaccharids, and mannite and dextrin among the polysaccharids. In the course of the work, the fermentive properties of about 502 strains have been determined. A number of duplicate strains were, of course, picked from the same plate during the examination of a given case, although an attempt was made to pick only colonies which showed apparent differences in growth characteristics. Likewise, certain cases have been studied over considerable lengths of time being frequently cultivated, and again similar strains were isolated from time to time. In the tabular summary of the fermentive reactions of the group, table 1, only case strains are included; that is, each strain
included in the table represents a particular variety of organism occurring in a given case and does not include duplicate strains isolated at the same time or in subsequent examinations. The reaction of 266 such case strains are presented. They represent organisms present in the nose and throat of normal persons and of persons suffering from influenza and from cases of common colds.

There are, on the basis of results presented in table 1, two main groups of gram-negative cocci present in cultures from the normal nose and throat, and in acute respiratory infection. One group of organisms includes those cocci which do not attack any of the carbo-

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Case Strains</th>
<th>Dextrose</th>
<th>Levu-lose</th>
<th>Maltose</th>
<th>Saccharose</th>
<th>Lactose</th>
<th>Galactose</th>
<th>Mannite</th>
<th>Dextrin</th>
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<tr>
<td>M. catarrhalis</td>
<td>98</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<td>M. catarrhalis Subgroup</td>
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<td></td>
</tr>
<tr>
<td>A</td>
<td>5</td>
<td>±</td>
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<td>-</td>
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</tr>
<tr>
<td>Meningococcus</td>
<td>8</td>
<td>±</td>
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<td>-</td>
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<tr>
<td>M. pharyngis sicus</td>
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<td>+</td>
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<td>-</td>
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<tr>
<td>Diplococcus crassus</td>
<td>15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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</tr>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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<td>-</td>
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<tr>
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<td>20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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<td>Chromogenic group 6</td>
<td>21</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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</tr>
</tbody>
</table>

* One strain fermented maltose as well as dextrose. Agglutination for meningococcus, negative.

Key: - = no fermentation; ± = very slight acidity in 24 hours, alkaline 7 days; + = distinctly acid; ± = variable reaction on polyescharides, but one or the other fermented or both.

hydrates, the Micrococcus catarrhalis group. The second general group is characterized by its chromogenic properties and may be divided into various subgroups on the basis of fermentation reactions. In addition to these two larger groups, meningococci have been isolated in a small percentage of cases. Other organisms encountered are M. pharyngis siccus and Diplococcus crassus. Diplococcus mucosus, observed by von Lingelsheim in a study of gram-negative cocci in epidemic meningitis was not encountered. In addition to the organisms classified in the preceding table, a few strains did not fall into any of the recognized groups. Two chromogenic strains produced a rather well marked red pigment in contrast to the greenish-yellow pigment produced by other chromogenic gram-negative cocci. Sugar reactions showed acid production from dextrose, levulose, saccharose, lactose and maltose.
Three other strains produced an intensely yellow pigment. Of these 3 strains, 2 fermented dextrose and saccharose only, while the third fermented dextrose, levulose and saccharose. These 5 organisms are grouped together in succeeding tables as heterologous strains.

The largest single group of organisms is that group distinguished by lack of acid-producing power when grown on the carbohydrates tested. This group has been termed the Micrococcus catarrhalis group. It has been found to be representative of a group of organisms rather than a single distinctive species. While all of the 98 case strains included in the tabular summary have shown the common property of lack of fermentive power, morphologic and especially cultural studies indicate that there is a distinct difference among various strains. This fact has been noted by previous workers, notably by Elser and Hunttoon who distinguished 2 cultural types of M. catarrhalis. Based on strains studied in this work, the members of the group can be divided into 4 rather well marked subgroups, all of which fail to ferment carbohydrates, with possibly a fifth, designated in the table as M. catarrhalis subgroup A, which differs from the rest in its reactions toward sugars. The largest subgroup among the nonfermenting types corresponds to the classical description of M. catarrhalis given by Ghon and H. Pfeiffer. The colonies appear after 24 hours as somewhat raised, opaque, white or gray-white disks, about the size of a meningococcus colony. When examined under the dissecting microscope, they are seen to have an irregular serrated margin and may be confused with colonies of certain diphtheroid organisms. The colony is typically granular, differing in this respect from the meningococcus colony which is more homogeneous. The different elements of the colony are strongly adherent to each other, and fragments may be detached with a needle. It differs in this toughness and tenacity of structure from most of the other types of gram-negative cocci. Morphologically the coccus is gram-negative and usually somewhat larger than the meningococcus. It characteristically appears in diplococcus form, although occasionally strains show numerous tetrads. Like most of the members of the group, it shows an early tendency to present degeneration forms, and even in young cultures, particularly in older ones, variations in size of different cells is readily observed. The cocci retain the Gram stain in some degree as swelling and degeneration progress. Irregularly stained preparations result, some cells being

gram-negative, others, especially the giant forms, appearing gram-
positive. These organisms grow readily in subculture and usually can
be maintained on starch agar for long periods of time without difficulty.
Occasionally certain strains exhibit a tendency to die out for no
apparent reason, even after a long period of cultivation on artificial
medium. Daily subcultures and use of blood medium are necessary to
restore satisfactory growth. Sixty-eight of the 98 case strains studied
belong to this division.

The second subgroup corresponds in all respects to the preceding
organism in colony structure, morphology and growth characteristics.
It differs, however, in that a pale yellow pigment is formed by cultures
grown on starch or plain veal-infusion agar. It was believed at first
that these strains must belong to the group of chromogenic cocci so
commonly found in the nose and throat, although the color of pigment
formed was not exactly characteristic of the latter, which is a greenish
yellow. Repeated transfers have been made to carbohydrate mediums
in the course of the study of these strains, but never have they shown
any indication of acid-producing power. Four such case strains have
been isolated. One was obtained from a normal case, cultivated
weekly for 3 months, with the coccus consistently present in all
examinations.

A third subgroup, including 18 like strains, showed little cultural
resemblance to the former 2. The colony differs markedly and indeed
has much the appearance of a meningococcus colony, except that it is
smaller. They are about one-half the size of the predominating type
of M. catarrhalis colony and are flat, gray, rather than whitish gray,
and translucent. Structurally, the colony is homogeneous, without
granular appearance, as a rule, although slight granulation may appear
toward the center. The surface is smooth and glistening while the
edges are regular, in contrast to the jagged outline of the more common
M. catarrhalis colony. In all respects it resembles a small, meningococ-cus colony. Morphologically the organism has practically the same
appearance as other forms mentioned. Growth of recently isolated
cultures is usually scant on primary isolation, but generally increases in
amount after continued cultivation. Growth at best was irregular,
however, and it was this group which gave the most trouble in main-
taining stock cultures. Growth characteristics remained unchanged,
however, with most of the strains, but 4 of them, after several months
on artificial medium, gradually presented an appearance more nearly
resembling the type of growth of the typical M. catarrhalis.
Another group of 8 strains differed a great deal from all other strains studied. The colonies were almost pinpoint in size, being extremely minute. They were transparent, glistening and possessed smoothly rounded edges. Such cultures, even when grown artificially for some time, have failed to show any change in characteristics, and maintain the same delicate growth. Cultures are initiated with great difficulty on other than blood agar, and to maintain them it has been necessary to transfer at weekly intervals, using blood medium. These strains are also distinct morphologically. Although derived from this fine delicate appearing colony, stained preparations of the coccus made after 24 hours' growth show the presence of a 'giant form of coccus with frequent metachromiasis. These stained preparations had all the appearance of a like preparation made from an old culture of the first type of M. catarrhalis, so much so that a series of stains were made from these cultures at early periods of growth, at 4, 6, 8 and 12 hour periods, to ascertain whether at these stages the organism might not present the usual morphology. At the 4-hour period the cocci were larger than the ordinary gram-negative coccus, but later at 8 and 12 hour periods the morphologic picture was much like that of the ordinary M. catarrhalis. Later the giant forms appeared. It would seem, then, that these strains represent a type of organism allied to the others, but undergoing more rapid transition into degenerative forms.

The last subdivision of the group consists of about 5 strains which agree exactly, in morphology and in growth characteristics, with the predominating type. They are different, in that they give rise to a slight degree of acid production on certain of the sugars—always dextrose, and maltose, as well in the case of one of the strains. The degree of acidity produced is slight, just sufficient to impart a delicate pink color to the medium. This acidity is transitory, however, and readings at 48 to 72 hours show a loss of color, with 7-day readings indicating a final alkaline reaction. It was felt that minute differences in the reaction of different lots of medium might account for the slight initial acidity, but repeated tests gave like results. Since observations have been based on 7-day readings, these strains have been classed as members of the M. catarrhalis group, and are indicated in the table as "M. catarrhalis subgroup A." It is quite probable that these strains belong to the same group, but represent a distinct variety.
The group of gram-negative cocci which are classed as M. catarrhalis can then be divided into 4 definite subgroups on the basis of cultural differences and to a certain extent on morphologic variation. An additional subgroup is recognized which is characterized by a slight initial acidity with certain carbohydrates, followed by a terminal alkalinity. All have the common property of spontaneous sedimentation when suspended in salt solution or nutrient broth, although that property is less marked in members of the third subgroup described.

A number of gram-negative cocci, possessing a common property of pigment production, but differing widely in fermentive properties, constitute a group second in incidence to the M. catarrhalis group. With the exception of chromogenic group 6, all of these organisms agree morphologically, and growth characteristics are for all practical purposes the same. Slight variations in the quality of the pigment are evident in different strains, but in general the colonies when viewed by transmitted light have a greenish yellow color and are semi-opaque. Viewed by reflected light, the color of colonies on agar appears to be greenish gray. They are somewhat smaller than colonies of the M. catarrhalis group, being about 1 mm. in diameter at 24 hours. Rather distinct variations in colony appearance are evident in different strains. Some of them closely resemble that of M. catarrhalis, while others, particularly strains belonging to chromogenic group 3, are quite like a meningococcus colony. The usual colony appearance, however, is that of an irregularly circular disk with more or less jagged outlines and a varying granular structure. Most of the colonies possess the dense, compact qualities peculiar to the M. catarrhalis colony and can be broken up into numerous crumbly fragments when touched with a needle. The organisms of chromogenic group 6 differ from the rest. The organism occurs usually in pairs or tetrads, but occasionally decided clumps are observed in stained preparations. Colonies appear more opaque than those of other chromogenic cocci and are larger. The pigment is usually a pale yellow and does not possess the greenish tint common to the rest.

On the basis of sugar variations the chromogenic organisms can be divided into 6 different groups. Three small groups ferment, respectively, dextrose only, dextrose and levulose, and dextrose and maltose. A larger group ferments 3 of the carbohydrates—dextrose, levulose and maltose. The largest group, constituting about one-half of the strains, having pigment producing power, ferments dextrose, levulose,
maltose and saccharose. Group 6 ferments uniformly all of the mono-
saccharids and disaccharids employed, and always one or the other of
the 2 polysaccharids, sometimes both. It is probable that members of
this group, judged by their morphology, growth characteristics and the
quality of the pigment produced, are in the light of the fermentation
reactions to be regarded as aberrant or degenerated forms of the
staphylococci. They were, however, consistently gram-negative and
are classed with the other chromogens. The probable relationship of
certain other types of gram-negative chromogens to the staphylococcus,
particularly organisms corresponding to the chromogenic group 5 of
this classification, has been pointed out by von Lingelsheim.

M. pharyngis siccus has been encountered a number of times. The
colonies of this organism are readily distinguished from other types
of gram-negative cocci by their decided firmness of structure. It is
almost impossible to break them up with a needle, and indeed the whole
colony can be lifted bodily from the surface of the medium. They are
closely adherent to the medium and about the size of the M. catarrhalis
colony. Morphologically the cocci occur in pairs, but are distinctly
smaller than other gram-negative cocci observed.

Diplococcus crassus, described in detail by von Lingelsheim, occurred in 10 cases of the series. It retains the gram stain with more
tenacity than other gram-negative cocci, and usually weakly gram-
positive and gram-negative members can be observed in the same prepa-
ration, especially in older cultures. The colony is small, grayish white
and rather compact.

Meningococci constitute the last division in the group. The mor-
phology and characteristics of growth are too well known to require
detailed description. It will be noted that chromogenic group 3 and
the meningococci have identical sugar reactions. In all cases, con-
firmation of meningococci has been made by agglutination with a
polyvalent meningococcus serum.

Analysis of this classification of gram-negative cocci by cultural
methods establishes the fact that the M. catarrhalis group has the
most common incidence in cases of common colds and influenza, and
in normal throats. Chromogenic cocci occur frequently, but the inci-
dence of any particular type is not marked. The chromogens have
been studied serologically by Elser and Huntoon who demonstrate
that organisms corresponding to our group 5, the most common chromo-
gen in the upper respiratory tract, are immunologically different,
although agreeing in fermentation. Strains corresponding to the present groups 2 and 3 were serologically the same. Further study of these organisms has not been attempted on account of the conclusive results of these workers, and because no group occurs with any decided frequency in cases of respiratory infection. It has seemed important to determine whether or not the different strains of *M. catarrhalis*, which have been found to occur so frequently, are representative of a single dominant type in this large percentage of colds and influenza and are capable of being distinguished from types of the same organism occurring in the normal nose and throat. For this reason a serologic study of representative strains has been undertaken.

Agglutination tests could not be used for, unfortunately, all of the strains with the exception of 8 of the cultural type 3, and 1 strain of the cultural type 1, had the undesirable quality of spontaneous sedimentation. Attempts were made to obtain stable suspensions by various means, such as variation of the salt content of the medium in which they were taken up, and by the addition of a minute amount of alkali or acid to the suspension, but without success.

The technic of complement fixation seemed to furnish the most practical method. Before undertaking a serologic classification by this technic, preliminary tests were made to determine the extent to which antibodies for *M. catarrhalis* could be developed in an experimental animal. Rabbits were injected intravenously with typical strains. Immunization is relatively easy for the animals will tolerate large initial doses of live organisms. Bacteriolytic experiments with immune serums, plus complement and a standardized suspension of cocci demonstrated that strongly lytic serums could be prepared. Serum for one of the agglutinable strains gave an agglutinin titer of 1:2,000.

Antigens were prepared for 15 different strains representative of the various cultural types, and cross-fixation experiments, following standardization of the antigens with polyvalent serums, have been undertaken. The results are as yet incomplete and will be reported in a later paper. Sufficient data are at hand to determine that the various strains which are alike on the basis of suar reactions are not representative of a single type of organism when judged by complement-fixation reactions. At least 3 different types can be differentiated. Whether the serologic grouping can be correlated with the 4 cultural types which have been described, cannot yet be determined.
THE GRAM-NEGATIVE COCCI IN THE NORMAL RESPIRATORY TRACT

The chief aim of this investigation has been directed toward a more exact knowledge of the relationship of these gram-negative coccal forms to acute infections of the upper respiratory tract. The work on classification was preliminary and essential to any interpretation of the significance of the organisms observed in various pathologic conditions. Definite knowledge of the incidence of the various groups of gram-negative cocci has been sought likewise in the normal nose and throat in order that a workable basis for comparison with the incidence under pathologic conditions might be available.

The normal carrier rate for the meningococcus has been the subject of extensive investigation during epidemic periods and depends largely on the extent of contact of the so-called normal population. In non-epidemic times the number of persons who carry meningococci in their upper respiratory tract varies, according to most authorities, from 3 to 6%, but in epidemic periods rises much higher. Frequent references in the literature to the presence of M. catarrhalis in disease conditions are available, dating from the original work of Ghon and Pfeiffer. No comprehensive study of the later organism in reference to its occurrence in the normal respiratory tract can be found. Arkwright\textsuperscript{15} made nasal examinations of 15 normal subjects and found organisms which he classed as M. catarrhalis in 5 cases, or 33%. No information concerning the incidence of the other varieties of gram-negative cocci under normal conditions could be found. A series of 6 normal persons were studied by Bloomfield\textsuperscript{16} at weekly intervals over a period of from 1 to 3 months. In practically all cultures of the series the predominating organisms were found to belong to the group of gram-negative cocci. No attempt was made to differentiate species other than the meningococcus. It seemed highly desirable to determine a normal incidence for all types, particularly a rate which would have been established by the same methods, and under the same conditions, as the subsequent study of respiratory conditions.

Various groups of normal persons have been studied at different times of the year in order to obtain an idea of the general incidence, as well as seasonal variations. One series was cultured during the spring months, April and May, a second during the summer months, June, July and Aug., while the third group was studied during Nov.,

\textsuperscript{15} Jour. Hygiene, 1907, 7, p. 145.
Dec., Jan., and Feb. The winter group includes cases from both 1919-20, and 1920-21. All of the subjects for study were drawn from the general student population at the university, and are probably representative of the ordinary urban community. No person in these groups had at the time of examination been knowingly exposed to colds, nor had they suffered from colds within recent date. A careful clinical examination was made before culturing to rule out possible inflammation of the respiratory mucous membrane. The results of the study of the nose and throat of normal persons are presented in table 2.

<table>
<thead>
<tr>
<th>Series</th>
<th>Number Examine</th>
<th>M. Catarrhalis</th>
<th>M. Pharyngis Siccus</th>
<th>Diplococcus Orosus</th>
<th>Meningoococcus</th>
<th>Chromogenic Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring--------</td>
<td>55 15</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Summer--------</td>
<td>47 24</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Winter--------</td>
<td>28 12</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Total………</td>
<td>110 51</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>Percentage incidence all cases…</td>
<td>46 9</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>17</td>
<td>7</td>
</tr>
</tbody>
</table>

This summary presents the total number of subjects included in each series and indicates likewise the number from which organisms of the various groups were isolated. The group which does not possess fermentive power, M. catarrhalis, constitutes by far the most common type observed in the respiratory tract. Various types of chromogenic cocci come next in order. M. pharyngis siccus is present in 9% of persons. The most significant fact brought out by these experiments, conducted as they were at different times of the year, is the practical absence of any seasonal variation. All of the organisms, including the most common group, M. catarrhalis, show practically the same percentage incidence at all times of the year. It was expected that the summer series might show a lower incidence than that observed during the colder months when respiratory infections are more prevalent. Such did not prove to be the case.

THE GRAM-NEGATIVE COCCI IN COMMON Colds

M. catarrhalis is the only organism among the group of gram-negative cocci, to which any importance has been ascribed by previous
workers, as a possible factor in the cause of infections of the upper respiratory tract of the type of common colds.

The organism when originally observed by Seifert,17 was found by him in the sputum and nasal secretions of persons affected by a small epidemic of infectious bronchitis. Later the organism was encountered by R. Pfeiffer 18 who obtained it in culture and gave it its name. He found it in large numbers in the bronchioles and alveoli in children with bronchopneumonia. Frosch and Kolle,19 in subsequent studies, found it in other cases of bronchitis. The organism observed by Ritchie 20 in a case of bronchopneumonia in a child was doubtless the same organism. Numerous early investigators who give indeterminate descriptions of “gonococcus-like organisms” or “meningococcus-like organisms” as encountered in the simpler respiratory infections were probably dealing with M. catarrhalis. Ghon and H. Pfeiffer14 made an extended study of the organism, which they found in numerous cases of acute bronchitis and bronchopneumonia. Lord20 has observed M. catarrhalis in the sputum of about 12 patients with ordinary bronchitis. Arkwright21 isolated this coccus from 11 of a series of 33 cases of catarrhal inflammation of the nose. Voorhies21 and likewise Mackay22 state that common colds are due to a number of organisms, among them M. catarrhalis. M. catarrhalis observed in most of the cases reported by these investigators has usually been associated with some other organism, such as the pneumococcus, the streptococcus, or the bacillus of Pfeiffer, although in a smaller number of cases, it has been found essentially as the only organism involved.

In the present study, investigation has not been confined to a determination of the prevalence of M. catarrhalis in acute upper respiratory infections, but an attempt has been made to determine the complete gram-negative flora. It seemed desirable to observe the degree of association of the other groups of gram-negative cocci with M. catarrhalis and with other organisms in respiratory infections, with the possibility in mind that they might exercise some symbiotic relationship, if no distinct pathogenesis. Our cases of colds have been obtained from the same general student population at the University of Chicago that furnished the subjects studied in the several normal series. No attempt has been made to select certain types of infection. All cases were examined as reported from time to time. Consequently the cases included in the “cold” series vary considerably in the clinical type of infection. A special effort was made to have cases available early in

the course of the inflammation. The majority were observed within the first 24 hours, rarely after a period of more than 48 hours following the onset of symptoms.

A total of 119 colds are included in the present series. A summary is presented in table 3 of the gram-negative cocci present, classified according to fermentation reactions, and subdivided according to differences in clinical diagnosis of the subject.

<table>
<thead>
<tr>
<th>Type of Infection</th>
<th>Number of Cases Examined</th>
<th>Micrococcus Catarhalis</th>
<th>M. Catarrhalis Subgroup A</th>
<th>M. Pharyngis Siccus</th>
<th>Diplococcus Cranitis</th>
<th>Meningo-coccus</th>
<th>Chromogenic Group</th>
<th>Heterologous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute rhinitis...</td>
<td>55</td>
<td>25</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Acute rhinitis...</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acute rhinitis...</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acute rhinitis...</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Acute rhinitis...</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acute pharyngitis...</td>
<td>14</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acute pharyngitis...</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Acute tonsillitis...</td>
<td>11</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acute tonsillitis...</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Epidemic tonsillitis... and pharyngitis...</td>
<td>16</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Acute laryngitis...</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total colds...</td>
<td>119</td>
<td>54</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Percentage incidence in all cases...</td>
<td>...</td>
<td>45</td>
<td>3</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

One striking feature is evident when the results obtained from the study of the gram-negative cocci in colds are compared with the figures derived from the study of normal persons. The incidence of the various groups which have been differentiated agrees almost exactly in both instances. The frequency of M. catarrhalis in the normal and the pathologic throat is essentially identical, 46% in normal persons and 45% in persons with various types of colds. Similar agreement exists for most of the other groups.
No decided variation in the incidence of various groups can be observed in the different clinical forms of inflammation which have been studied. If one may compare those cases in which the infection was confined to the nose or pharynx with the fewer cases which presented bronchial involvement as well as inflammation of some portion of the respiratory tract higher up, it would seem that gram-negative cocci, particularly M. catarrhalis, occur less frequently in the latter type of cold.

In the course of the work, no attempt was made to determine the exact numbers of gram-negative cocci in given plate cultures in relation to members of the pneumococcus, streptococcus, staphylococcus and Pfeiffer bacillus groups. Rough plate readings were made, however. While colonies of the general gram-negative character could be observed in the majority of cultures—somewhat more than three-fourths of them—nevertheless it was uncommon to find the gram-negative flora constituting more than 10% to 30% of colonies. Occasionally plate cultures would be dominated by these organisms, sometimes constituting from 80% to 90% of all colonies.

THE GRAM-NEGATIVE COCCI IN INFLUENZA

A recurrent epidemic of influenza of short duration occurred during the early part of 1920, following the pandemic of 1918. The work which we were doing on common colds was suspended and our investigations were confined to a similar study of the incidence of gram-negative cocci in influenza. Only a relatively small number of cases could be investigated, due to the limited time that material was available. Three different groups of cases were studied, all occurring within the vicinity of Chicago. It was felt that if cases could be studied which developed in different localities where contact between groups was unlikely, that possibly the comparison afforded might permit a generalization on the bacteriologic conditions in this type of infection.

The presence of M. catarrhalis in influenzal conditions was demonstrated as early as 1890, when Kirchner 23 first reported it as being one of the organisms encountered in influenza, prevalent at that time in epidemic proportions. Subsequent to the epidemic of 1890, several instances are on record in which the organism has been found in inter-epidemic outbreaks of influenza-like infection. In certain instances the

organism has been observed in connection with certain others of the common respiratory micro-organisms, such as the pneumococcus or the streptococcus. At other times, it has been considered by various writers as the chief organism involved in particular outbreaks. The studies of Ghon and H. Pfeiffer have been referred to. In 1905, Dunn and Gordon record an epidemic simulating influenza in which M. catarrhalis seemed to be the predominating bacterium. Bezanson and De Jong describe an epidemic occurring the same year in Paris, with M. catarrhalis dominant in bacterial cultures.

In the most recent pandemic of influenza, a great number of workers found M. catarrhalis in cases distributed throughout the country. Practically every bacteriologic study contains some reference

<table>
<thead>
<tr>
<th>Case Series</th>
<th>Number of Cases Examined</th>
<th>Micrococcus</th>
<th>M. Catarrhalis</th>
<th>M. Pharyngis</th>
<th>Diplococcus</th>
<th>Meningococcus</th>
<th>Chromogenic Group</th>
<th>Heterogenous</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Chicago</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1 2 3 4 5 6</td>
<td>0</td>
</tr>
<tr>
<td>Great Lakes</td>
<td>20</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0 0 1 1 0 2 3 0</td>
<td>0</td>
</tr>
<tr>
<td>Camp Grant</td>
<td>12</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0 0 0 0 0 1 0 0</td>
<td>0</td>
</tr>
<tr>
<td>Total cases, percentage incidence of all cases...</td>
<td>40</td>
<td>14</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0 0 2 1 4 3 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>3</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>0 0 5 3 10 8 0</td>
<td>0</td>
</tr>
</tbody>
</table>

to the organisms, although no detailed study of its relationship to influenza has been encountered. No attempt will be made to summarize the work of the past few years. The general deduction may be drawn that while M. catarrhalis is one of the more common species encountered in epidemic influenza, it rarely occupies a dominant position in the bacterial flora. Little importance has been accredited this organism as a factor in the pathogenesis of influenza. Finally, Clark and Murphy have reported M. catarrhalis as the predominating organism in a recurrent epidemic occurring at the same time as the present group of cases.

The group of cases included in this report is derived from three sources. A number of the cases developed among the same general

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student population at the University of Chicago, from which we had been drawing our material for the study of common colds. A group of cases occurring at the Great Lakes Naval Training Station, Waukegan, Illinois, constitutes our second series. The third series was obtained from soldiers stationed at Camp Grant, Rockford, Ill. The results of the examinations from these three series of influenza cases are contained in table 4.

The total number of cases that it was possible to examine during the brief course of the epidemic is too small to warrant definite conclusions. The significant feature is the drop in the general incidence of gram-negative cocci. All forms, including M. catarrhalis, occurred less frequently in influenza cases than in common colds. Curiously enough, the incidence of M. catarrhalis was found to be lower in cases of influenza than in the group of normal persons. It would seem that in these cases at least, the gram-negative forms had been crowded out or overgrown by some more virulent organism, judged by the normal incidence level established in previous studies.

**AN ATTEMPT TO DETERMINE THE SPECIFIC RELATIONSHIP OF MICROCOCCUS CATARRHALIS TO COMMON COLDs**

Experimental data have been presented which furnish evidence of the presence of M. catarrhalis in a large percentage of common colds and influenza. The same group of organisms, however, has been found in normal persons just as frequently. If any significance was to be attached to the presence of this organism in the pathologic nose and throat, it seemed necessary to investigate certain possibilities. It was necessary to determine, whether in given cases of colds, M. catarrhalis had been present on the mucous membrane as a normal inhabitant previous to the development of the infection. If it were present for some time before the onset of symptoms, did it increase perceptibly in numbers with the developing infection, and thereby exercise a possible symbiotic relationship with the real causative agent of the inflammation? There was also the possibility that certain colds might be encountered which would show no previous carrier condition of M. catarrhalis, but presence of the organism during the infection. In such cases, it was important to determine whether the organism gained entrance into the inflamed area coincidently with developing symptoms, previous to that time, or somewhat later in the course of the infection. If these points could be settled, more exact data regarding the specific relationship of M. catarrhalis would be available.
With these ideas in mind, a group of 10 normal persons was selected, and each one subjected to routine daily cultures over a period of 2½ months. Each subject was watched closely for the onset of symptoms which would indicate an oncoming cold. Following the initial examination, detailed studies of each culture were not made, except when colds developed. The routine procedure consisted of a critical examination of the plates each day, when an estimated percentage was recorded of the number of different types of colonies which had developed. This included the 4 more important groups, the green-producing colonies, the Pfeiffer-like colonies, staphylococcus, and gram-negative-like colonies. From time to time, at intervals of perhaps 2 weeks, detailed studies were made of gram-negative-like colonies in order to maintain a general idea of the specific species comprising that flora.

During the course of these experiments, 5 of the 10 subjects in the series developed one or more colds, the other 5 remaining normal throughout the period of observation.

Gram-negative cocci were isolated from all of the subjects who remained normal, at some time during the course of the experiments. They were present in variable numbers in practically all plate cultures during the series. Only 2 of them, however, subjects A7 and A5, gave cultures of M. catarrhalis while under observation.

Subjects A1, A2, A3, A6 and A9 developed colds at different times during the experiments. Subject A1 showed gram-negative cocci of the Diplococcus crassus type when first examined, with M. catarrhalis absent. Gram-negative-like colonies were not numerous. An acute pharyngitis developed about 2 weeks later, at which time M. catarrhalis was identified and gram-negative-like colonies constituted 45% of total colony development from swabs. Following the cold, catarrhalis-like colonies dropped to about 10 or 15% of all colonies. A little more than a month after the first pharyngitis, a second inflammation and cough were observed. Again catarrhalis-like colonies increased in relative numbers, beginning noticeably the day before symptoms appeared, when they constituted 50% of all colonies. On the day that the cold was apparent, 30% of gram-negative-like colonies were present, the next day 40%, and the second day, 80%. M. catarrhalis was demonstrated at this time and likewise 4 days later. Following the cold, the incidence of gram-negative-like colonies fell to a level of from 10 to 20%, with M. catarrhalis unable to be demonstrated.
The initial examination of subject A2 showed a considerable percentage incidence of gram-negative-like colonies. The organisms were found to belong to chromogenic group 5. On the second day after initiation of study, an acute rhinitis developed. M. catarrhalis could not be demonstrated. Three weeks after the first cold, a second rhinitis was diagnosed. Gram-negative-like colonies were few in number, almost negligible, and these few were the same chromogen 5. On the third day of the infection gram-negative-like colonies had increased in number to 30% of the total number of colonies, and M. catarrhalis was isolated, together with the chromogen. They continued to increase in numbers, and the next day the group represented 50% of the flora.

The history of subject A3 resembles that of A2 as regards incidence of colds. An acute rhinitis developed the second day of observation. Large numbers, 50%, of chromogen 5 organisms were observed early in the infection, and indeed colonies of the gram-negative type dominated the plates. M. catarrhalis was not isolated until 8 days after the beginning of symptoms, and its presence was transitory as it was negative 2 days later and continued to remain so. A second rhinitis developed about 6 weeks later, and although gram-negative-like colonies showed an increase in numbers, M. catarrhalis could not be found.

Subject A6, at the beginning of the study, had a low incidence of gram-negative-like colonies which were of the chromogenic groups. An acute rhinitis developed in 3 weeks, but no marked increase in gram-negative organisms could be observed, and M. catarrhalis was absent. M. catarrhalis was only isolated once during the study of cultures from this subject. Almost a month after the termination of the cold, there was a sudden flare-up of catarrhalis-like colonies with a colony percentage incidence of 85%. M. catarrhalis was identified. This marked increase was transitory, the number decreasing shortly with M. catarrhalis absent in cultures thereafter.

The gram-negative cocci were apparently of little importance in the history of subject A9. They could be identified in most cultures as constituting about 10% of the flora. M. pharyngis siccus and group 5 chromogens were identified. M. catarrhalis was isolated at no time, and although a mild pharyngitis developed 3 weeks after the start of the study, no relative change could be observed in the gram-negative flora.

Analysis of the 5 cases in which colds developed brings out certain facts. A number of colds are encountered with which M. catarrhalis
is concerned in no way. Although gram-negative cocci could regularly be isolated during the extended examinations from subjects A3, A6 and A9, they apparently were concerned in no way with the colds which developed. M. catarrhalis was demonstrated in both subjects A3 and A6 but could not logically be connected in any way with the colds which were observed. A particular point of interest observed in subject A6 was the sudden and marked increase in gram-negative forms which occurred without apparent effect on the mucous membrane. M. catarrhalis constituted 85% of all colonies present on plates at that examination. Such transitory domination of the flora by a particular organism seems to demonstrate the frequently changing character of the bacterial flora in at least a considerable proportion of persons. Seemingly, a sudden dominant position in the flora can be attained by several organisms, possibly through invasion of the respiratory mucous membrane by a strain more virulent than its fellows. Such instances have been observed for the pneumococcus, Bacillus mucusus capsulatus and the Pfeiffer bacillus as well as M. catarrhalis, in a study of normal throats over extended periods.

The two cases of rhinitis which occurred in the study of subject A1 influenced certain changes in the gram-negative flora. M. catarrhalis had not been present in cultures previous to the infection. Increased numbers of gram-negative-like colonies were observed the day before symptoms and during the height of the infection. Gram-negative forms, including M. catarrhalis, were distinctly dominant in plate cultures. With subsidence of the infection the organisms tended to disappear. The tremendous increase in numbers may have been due to the acute process so altering the environment that the organisms took hold and grew more rapidly at the seat of the disease. In the rhinitis of subject A2, M. catarrhalis, which was observed late in the cold, can probably best be interpreted as one of the secondary invaders.

Eight colds, then, have developed in these 5 persons. M. catarrhalis was apparently concerned in no way with 5 of them. In one, it seems to have invaded secondarily the previously inflamed area. Two cases may have been caused by M. catarrhalis. It has been emphasized in a previous paper, however, that one cannot ascribe a causative relationship to a given organism, even when the evidence is reasonably favorable, for there is no direct knowledge of the period of incubation of such infections. The presence of M. catarrhalis at the time of infection may have meant merely that under the stimulus of the true exciting
agent of the cold, these organisms, normally in the throat, although
present in such small numbers that they could not be demonstrated,
rapidly began to multiply, and possibly acquired as well a distinct patho-
ogenicity. Thus one cannot determine whether the presence of the
organism indicated a direct or merely symbiotic relationship to the
infection.

THE EXTENT OF THE CARRIER STATE FOR MICROCOCCUS
CATARRHALIS

Organisms of the type of M. catarrhalis have been demonstrated
in essentially the same relative number of throats, whether they be
normal or pathologic. Such being the case, interest is attached to the
question of whether the organism is to be considered as a permanent
inhabitant of the throat, with such frequent occurrence, or whether
the presence of the organism in a given throat is limited, and its status
subject to frequent change.

M. catarrhalis does not appear to be carried in appreciable numbers
in throats of convalescents from colds. No general deduction can be
made from the few cold cases in the "A" series, in which M. catar-
rrhalis was involved. It was found in these cases, however, that M.
catarrhalis was carried a relatively short time following convalescence.

More important, particularly in view of the common incidence of
M. catarrhalis, is the relation of the organism to the normal person.
Is the organism present for a long period of time in a given throat; is
this the usual occurrence in a considerable proportion of normal people;
and is it therefore most logically to be considered as a normal throat
saprophyte? Or, on the contrary, does it inhabit a given throat for
brief periods of time with the probability of frequent transfer from
person to person?

Three different groups of normal persons have been studied at
different times of the year. Cultures from the individuals of each
group were taken at weekly intervals over a period of 2 months, from
one group for 2½ months. The first group of 5 persons was studied
during the winter months of Nov., Dec. and Jan. No complicating
colds developed during the period of observation. Three of the 5
subjects did not show M. catarrhalis. In fact gram-negative-like
colonies were in the minority in practically all of the plates. The types
of organisms of the gram-negative group which were represented were
members of the chromogenic group, with one case showing M.
pharyngis siccus.
The fourth subject of the group possessed a throat flora which was consistently dominated by members of the gram-negative group. The percentage of colonies on the various plates, which were representative of gram-negative cocci, varied from 40 to 90%, and the usual finding approached the higher figure. M. catarrhalis was the chief organism among the gram-negative-like colonies, but chromogenic cocci were also isolated.

The flora of the last member of the group, subject 5, was likewise characterized by preponderance of gram-negative-like colonies over other forms. This condition continued with periodic variations throughout the period of observation. The flora consisted of M. catarrhalis as well as a chromogenic coccus of group 5.

A different group of 6 persons was studied in the same way during the Spring months, April, May and early June. Two of them were negative for M. catarrhalis during the experiments, although the familiar chromogens were observed in one. Three subjects, NC 56, NC 154 and NC 505, gave positive cultures of M. catarrhalis throughout the period of observation. The other subject of the series, NC 228, harbored M. catarrhalis in the upper respiratory tract at the initial examination and for one month thereafter, following which cultures proved to be negative.

The third study of normal persons was conducted during the summer months, June, July and Aug. Five persons constituted this group. In cultures from 2 of them, S2 and S5, M. catarrhalis could not be found at any time. Chromogens were present in one case, M. pharyngis siccus in the other, while the latter likewise gave positive cultures of the meningococcus for 3 successive weeks, after which that organism disappeared. The other 3 subjects, S1, S3 and S4 all showed consistently cultures of M. catarrhalis. Subjects S3 and S4 gave positive cultures throughout the period of observation. Cultures from subject S1 failed to reveal the presence of M. catarrhalis at the initial examination but were positive thereafter until the work was terminated.

These separate groups of persons, studied during different seasons of the year, have given results which furnish rather conclusive evidence of the rôle played by the group of gram-negative cocci, and particularly by M. catarrhalis. The case incidence of the catarrhalis group, considering the various series of persons as a unit, corresponds to the same general percentage observed in the larger series of normal persons on whom a single examination was made. All subjects who
showed M. catarrhalis in throat cultures were, with a single exception, carriers of that organism throughout the various periods of observation. The one exception was positive at the beginning of the study but became negative for M. catarrhalis after one month. The previous length of the carrier state was of course indeterminate. One feels that this group of organisms, M. catarrhalis, constitutes a species which has become highly acclimatized to the mucous membrane of the upper respiratory tract. A large proportion of persons carry it in the throat for long periods of time without visible effect on the lining membrane. Rare instances may arise in which a more virulent strain may be involved in respiratory infection. In general it would seem to lead a saprophytic existence in numerous throats, a condition comparable to that of the commonly recognized saprophytes of the mouth.

COMPARATIVE VIRULENCE OF STRAINS OF MICROCOCCUS CATARRHALIS FROM NORMAL SOURCES AND FROM Colds

M. catarrhalis has been demonstrated to be present in normal persons and in cases of colds with about equal frequency. In persons during influenzal infections it has been found less frequently than in normal persons. If any pathogenic properties are to be conceded the organism, it would seem essential to establish, among other things, a relatively heightened virulence of the coccus, when found in cases of colds and influenza.

Various strains have been tested in regard to their virulence for mice. These strains have been derived from different types of respiratory infection and from normal persons. While no definite information was at hand, it has been assumed that M. catarrhalis possesses the characteristic, common to so many bacteria, of a decline in virulence following continued cultivation on artificial medium. The following uniform procedure has been followed, in order that virulence might be tested as soon as possible after recovery of the strain from the throat, and likewise that directly comparable data might be obtained. Colonies were picked from blood-agar plates to blood-agar slants. Purity of culture was established by morphologic examination. Transfers were then made to dextrose medium, and if fermentation was not produced in 24 hours, a transfer was made from the original blood-agar slant to a second tube of the same medium. This 24-hour old culture was employed for the virulence tests. It was consequently in
its third generation on blood agar. By this method conditions were exactly comparable in all instances. Classification of the organism was verified later by the usual methods.

In preparing cultures for inoculation, care was taken to seed as nearly as possible the same extent of blood-agar medium. Growths were washed off in 2 c c of warm sterile broth and mice were inoculated intraperitoneally at once. In order to obtain an idea of the usual lethal dose for mice, preliminary tests were made with 4 strains from cases of colds. Mice were inoculated, respectively, with one-eighth, one-fourth, and one-half of the slant culture of the first strain. None of the mice died within 6 days. The next 3 cold strains were tested by inoculating one-eighth, one-fourth, one-half and one slant into a series of mice. Death occurred in 24 hours in all 3 animals receiving the massive doses of 1 whole slant. One animal injected with one-half slant died in 3 days, strain C557J. All others survived. It was felt that the use of such tremendous doses as one full slant, with an attendant volume of 2 c c did not constitute a test of any value when inoculated into so small an animal as the white mouse. In subsequent determinations, each strain was injected in doses of one-fourth and one-half slant. Eight normal strains have been tested and 8 cold strains. Death was produced by 2 of the normal strains, S3D and 505A2, in one-half agar slant doses. All other animals survived. Three of the cold strains produced death, C629D influenza, C557J rhinitis and bronchitis, and C82H16 rhinitis, at periods of 4 days, 3 days, and 2 days, respectively. The lethal dose of strains C629D and C557J was one-half slant, of strain C82H16 one-fourth slant. The larger dose of this strain, curiously enough, did not kill the animal. Profound symptoms of toxemia were evidenced in all of the animals receiving the larger dose and usually in those receiving the smaller. Necropsy cultures from the mice which succumbed failed to reveal the organism in the heart blood. Three cases C557J, C82H16 and S5D, gave cultures from the peritoneal fluid, C557J from the pleural fluid as well. The only pathologic changes which could be observed were a variable increase in the amount of peritoneal fluid, enlargement and hyperemia of the spleen, occasional subserous hemorrhage in the intestines and in one case increased pleural fluid. There were no indications of a generalized infection, pathologically or bacteriologically.

The organism possesses little or no virulence for mice. Tremendous doses were required to produce death which was evidently due
more to the toxic effect of the bacterial protein than any growth and reproduction of the organism in the tissues. Furthermore, no difference could be determined between the lethal effects of strains isolated from normal and from cold sources.

Various strains studied were practically avirulent for rabbits. No definite experiments were conducted to determine the degree of virulence, but in immunizing animals against numerous strains, doses of one-fourth blood-agar slant of live organisms were commonly employed, intravenously as the initial dose, followed by one-half and one whole slant on the next two days, without ill effect.

DISCUSSION

Cocci which fail to retain the Gram stain constitute one of the more common groups of bacteria which may be found in the upper respiratory tract of man in both health and disease. They can be divided on the basis of fermentative power and growth characteristics, into several different groups. The largest group encountered in this study is made up of those organisms which fail to produce acid through action on any of the more common carbohydrates. These organisms have been recognized as constituting a single group, M. catarrhalis which, however, may be divided into 5 subgroups on the basis of cultural differences. A second group of organisms, characterized by the production of a greenish yellow pigment, occurs in the human respiratory tract with about the same frequency as the first group. These strains likewise may be divided into 6 different subgroups on the basis of differences in fermentation. The meningococcus of Weichselbaum, Diplococcus crassus Kral, and M. pharyngis siccus complete the list of gram-negative cocci found.

The essential purpose of this study was to investigate the relationship which these gram-negative cocci bear toward upper respiratory tract infection. Certain data have been obtained. Some member of the general group can be found in three-fourths or more of all cases, M. catarrhalis in about half that number. The percentage of incidence of all groups is, however, essentially the same in normal as in pathologic throats. In fact, they are less frequent in persons with influenza than in normal persons, an indication that they have been crowded out by a more virulent species. No single group of gram-negative cocci, other than M. catarrhalis, occurs with special frequency in common
colds and influenza. The variable and irregular occurrence of these other groups would seem to limit any importance possessed by gram-negative cocci to the rôle played by M. catarrhalis.

Extended study of certain normal persons found to harbor M. catarrhalis in the throat has led to the conclusion that the organism is decidedly a permanent inhabitant of the throat, rather than a temporary invader. It lodges in a considerable percentage of throats, becomes readily acclimatized to that environment, and lives there for long periods of time, leading apparently a saprophytic existence. Finally, various strains from normal sources and from colds have been found to be avirulent for white mice and rabbits. Lethal effects have been obtained with a few strains, but only when massive doses were employed. Strains from normal persons and from cases of common colds present, moreover, no distinguishable differences in lethal power.

Rarely, circumstances occur in which it may be possible to place some emphasis on M. catarrhalis in explaining the pathogenesis of a given cold. Such an instance has been discussed in connection with the two rhinites observed in subject 1 of the “A” series.

Observation of M. catarrhalis in normal throats and in various respiratory infections, justifies but one conclusion. The organism is only rarely involved in the pathogenesis of acute infections of the upper respiratory tract in man. Based on its like incidence in colds and in normal persons, the actual decline in incidence in influenza, its long contained existence on the mucous membranes of many normal persons, and finally its lack of virulence, irrespective of origin from normal or pathologic throats, the logical deduction is that its usual sphere is that of a harmless saprophyte.

CONCLUSIONS

Gram-negative cocci which occur in the nose and throat normally and in acute upper respiratory infections may be grouped according to cultural characteristics and fermentative differences. Such a classification is presented in table 1 and the succeeding discussion.

No essential difference was distinguished between the incidence of the various groups of gram-negative cocci in common colds, and in a like series of normal persons. In epidemic influenza, the incidence was less than in normal persons.
M. catarrhalis, the most common member of the group, is carried for long periods of time in the throats of many normal persons, constituting a permanent member of the normal throat flora.

No distinguishable differences in virulence for mice and rabbits could be determined between strains of M. catarrhalis from normal sources and those from colds or influenza.

These observations do not indicate that Micrococcus catarrhalis is generally concerned in the pathogenesis of common colds or influenza.