The work on the pneumococcus at the Boston City Hospital, under the auspices of the Commission for the Investigation of Acute Respiratory Diseases, of the Department of Health of the City of New York, was begun November 1, 1904, and continued until July 1, 1905. The director of the hospital laboratory, Dr. F. B. Mallory, encouraged us to accept the proposals of the Commission, placed the facilities of the laboratory at our disposal, and has maintained a constant interest in the progress of the investigation. The physicians of the hospital's clinical services, both visiting staff and house physicians, have given us every assistance in procuring material. On the part of the Commission we have had the support and advice of Dr. Theobald Smith. The photomicrographs we present were taken for us by Dr. S. B. Wolbach. To all of these gentlemen we extend thanks for their interest in the work and their invaluable assistance.

The purpose of the investigation was to ascertain, so far as possible with the material under our control, the distribution of the pneumococcus, and by a subsequent comparative study of cultures isolated to see if there might be a constant variation in any character or set of characters coincident with the source of origin. We wish to report as concisely as possible the data which bear directly on these questions.

One of the constant difficulties which has confronted us throughout the work has been the separation of the pneumococcus from Streptococcus pyogenes. The inulin test of Hiss having been proposed as an absolute method of differentiating these two species, we give in detail our experience with it in the form in which it was originally proposed and in the modification we find most satisfactory.
Studies on the Pneumococcus

In the course of the work we have twice encountered a diplococcus which has proved identical in general characters with those isolated by Howard and Perkins,¹ and by Richardson² in this country and by several observers abroad. It has usually been described under the name Streptococcus capsulatus or mucosus. As we have been able to add some new facts to those previously reported, we give our experience with it in some detail.

Except for the method of using the inulin, our technique is a combination of methods previously in use and well known to investigators who have studied this group of bacteria. We therefore make the briefest possible statement of it.

METHODS.

In cultivating the pneumococcus we have found that attention to the composition and reaction of the culture medium is essential. All media having bouillon as a base should be made from beef. The reaction should be adjusted with but one change, if possible, to between 0.4 and 0.8 % acid to phenolphthalein (cold titration). Dextrose, or some other carbohydrate fermentable by the particular culture in question must be present in quantity of from 0.5 to 1 %, or growth will be neither constant nor abundant. The addition of from 2 to 5 % of "fresh" defibrinated blood to dextrose agar-agar we have found to make the most satisfactory medium for routine cultivation. If these conditions are fulfilled, the temperature has only its usual influence on rapidity of growth. Growth always takes place at room temperature. The gelatin-agar mixtures with the addition of dextrose give good growth. The other culture media which we have found particularly useful for cultivation and identification of the organism are litmus-milk and the serum-water-sugar mixtures made according to the formula of Hiss. The lactose-serum water is particularly useful. It is coagulated by both the pneumococcus and the streptococcus as a rule, but by the former in twenty-four hours, and by the latter only after many days. It cannot be considered to be an absolutely differential medium.

In making isolations we have placed our dependence on the plating method with surface seeding. Dextrose agar-agar plates are poured and solidified. Several drops of sterile defibrinated blood are scattered over the surface with a finely drawn pipette. The particular kind of blood used seems to be unimportant. With a sterile platinum loop, several drops of a previously made bouillon or salt-solution suspension of the material to be plated are added to one of the drops of blood on the plate. After mixing thoroughly with the loop, a drop or two are carried to a second drop of blood, and after mixing again, to a third. After these dilutions are accomplished, the drops are spread as evenly as possible over the plate. If the agar-agar is firm (1.7 to 2 %), and the wire loop soft, this can be accomplished without roughening the surface of the

¹ Jour. of Med. Research, 1901, vi, 163.
² Jour. of the Boston Soc. of the Med. Sciences, v, 499.
medium. Colonies to be studied are transferred from the plates to the dextrose blood-agar. The isolation from the heart's blood of an animal after subcutaneous or intraperitoneal inoculation of the material to be examined is less satisfactory as a routine method, in our experience. If used with discretion, the method does not fail, but it is more tedious and expensive. We will say more of the method in discussing the virulence of the pneumococcus.

The identification of the pneumococcus cannot be made by any one feature or test. It is best made within a few days after isolation of a culture. The capsule is then more certain to be large and distinct, and the lanceolate diplococcus form is more marked. As the period of cultivation grows longer, the capsules are less constantly demonstrated. The cocci become smaller and the pairs more flattened. Under certain conditions which we do not fully understand, but which seem to be generally unfavorable to active growth, the most typical pneumococcus culture may grow as pairs of oval and flattened cocci in long chains. Many individual cocci in such a chain will appear distinctly biscuit-shaped. Figure 4, however, is intended to demonstrate that very long chains do occur even under favorable conditions with freshly isolated pneumococcus cultures. In such cases the size of the capsule, the larger size of the individual coccus, and the shape of the pairs must be depended on for the diagnosis.

The colony on blood-agar varies under conditions we have not been able to control, from an almost invisible dry, flat colony, or a raised, moist, or dewdrop-like growth, to a dead white, viscid colony 2 mm. in diameter, resembling that of Staphylococcus albus. Not much dependence can be placed on such a variable character in identifying a culture.

Culturally the rapid fermentation of lactose in serum water, the rapid coagulation of litmus milk, and the fermentation of inulin are the points on which most dependence must be placed, although any one of these characters may be absent with a given culture. If inulin be fermented by a micro-organism having in general the characters of the pneumococcus, the identity is established, in our experience. That there are, however, non-inulin-fermenting pneumococci, we feel certain. Such cultures will be described when we discuss the inulin test.

OCCURRENCE AND DISTRIBUTION OF THE PNEUMOCOCCUS.

In the study of the distribution of the pneumococcus, material was studied from:

I. Autopsies in the hospital.
II. Surgical material sent to the laboratory.
III. Cerebro-spinal fluid obtained by lumbar puncture.
IV. Sputum of—
   A.—Pneumonia cases.
   B.—Other cases with bronchial or pulmonary trouble.
   C.—Saliva from persons in good health at work in the hospital.

I. Autopsies.—The efforts of this laboratory have been chiefly directed to the study of autopsy material. We will report the
results of the bacteriological examination of the lungs of thirty-three persons dying with acute lobar pneumonia. We include also the bacteriological report of examination of the lung in four cases in which death was ascribed to lesions outside the lung, although the lungs showed areas of broncho-pneumonia, and in two cases in which there was no evidence of pulmonary disease.

In order that the value of the results may be correctly estimated, some explanation is necessary. Early in the course of the work our only aim was the isolation of the pneumococcus. Thus all of the examinations reported bear on the question of the general distribution of the pneumococcus. All of the cases in which the pneumococcus was the only bacterium isolated from the lung belong to this period when we were attempting nothing but its isolation. No consideration should be given to the negative aspect of these cases.

When the local distribution of the pneumococcus in the lung is considered, only two of our cases are of importance. These are reported in detail (Autopsy 1905, 47, and Autopsy 1905, 82).

The reported data which have reference to the association of bacteria in the pneumonic lung are not complete. Other microorganisms were found which were only roughly classified and noted. Especial effort was later made to isolate the streptococcus. In general, the negative character of the findings has no significance. There is one particular exception. The influenza bacillus has been kept constantly in mind and our technique has been suitable to grow it. We have, however, met with it only once (Autopsy 1905, 82), and the detail of that case is given.

The facts which concern the general distribution of the pneumococcus, the constancy with which it occurs in the pneumonic lung, its association with other bacteria in the pneumonic lung, and its presence in the lungs of persons dying of other causes than acute lobar pneumonia are presented in the following table:

(a) Cases in which the pneumococcus alone was isolated from the lung:
   - Acute lobar pneumonia: 5 cases.
   - Acute pericarditis: 2 cases.
   - Acute meningitis: 1 case.

The cases of pericarditis and meningitis showed areas of broncho-pneumonia.
(b) Cases in which the pneumococcus was isolated together with the streptococcus:
- Acute lobar pneumonia .......... 22 cases.
- Wood-alcohol poisoning ............ 1 case.
- Chronic interstitial nephritis ...... 1 case.

The cases of poisoning and of nephritis showed no lesion of the lung.

(c) Cases in which the pneumococcus was isolated together with: (1) Streptococcus pyogenes and (2) Pneumo-bacillus of Friedländer:
- Acute lobar pneumonia ............. 4 cases.
- Cerebral hemorrhage with broncho-pneumonia .......... 1 case.

(d) Cases in which the pneumococcus was isolated together with: (1) Streptococcus pyogenes, (2) Staphylococcus aureus and albus, (3) Influenza bacillus, (4) Pseudo-diphtheria bacillus:
- Acute lobar pneumonia ............. 1 case.

(e) Cases in which the pneumococcus was isolated together with: (1) Streptococcus pyogenes, (2) Pneumo-bacillus of Friedländer, (3) Streptococcus mucosus:
- Acute lobar pneumonia ............. 1 case.

After the work was well under way, it became apparent that the pneumococcus was present with great constancy in the pneumonic lung, and that it often occurred in the healthy as well as in the involved portions of the organ, usually in association with the streptococcus. It was realized that more careful study of the localization of pneumococci and streptococci in the lungs of pneumonia cases was essential to a consideration of the relationship of these micro-organisms to the disease and to one another. To answer these questions the examination of material from the lungs of patients recently dead was necessary. Decisive data might be expected from cases in which death occurred in an early stage of the disease. In the latter part of the winter we have had two cases in which these conditions were approximated. The records are reported in detail.

One of these cases (Autopsy 1905, 82) was unusual in that B. influenzae was present in very large numbers. From its peculiar interest in this respect the clinical history is reported.

**Case I.—Acute lobar pneumonia.** Autopsy 1905, 47, was performed nine hours after death. There was late red and early gray hepatization of the right lower lobe. All other lobes seemed normal. Twenty-six plates were made from the upper, middle, and lower parts of each lung; 1% glucose-agar with rabbit's blood was used and the plates were seeded on the surface. Suspensions of same number of loops of material from each portion of the lung were employed.
All the plates showed some colonies of Staphylococcus aureus and all showed the pneumococcus and the streptococcus in about equal numbers as estimated by the microscopic examination. Colonies were transplanted and cultures identified as shown in the accompanying table.

<table>
<thead>
<tr>
<th>Lobe of Lung</th>
<th>Pneumococcus</th>
<th>Streptococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonic right lower lobe</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Healthy right upper lobe</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>&quot; right middle &quot;</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>&quot; left lower &quot;</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>&quot; left upper &quot;</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

**Case II.**—E. H. B., aged forty-five, was admitted to the Boston City Hospital, May 3, 1905. Clinical diagnosis: lobar pneumonia. On admission the patient was delirious. A member of his family stated he had been sick for one week. There was considerable prostration, marked cyanosis and dyspnoea. He was delirious and required restraint. The eyes, nose, throat, and ears were negative. The chest was symmetrical. The heart sounds were indistinct and a friction rub was heard over the base of the precordium. The left lung revealed dulness from the angle of scapula to the base, extending into the axilla. The spleen was not felt. The abdomen was tympanic but not tender. The temperature on admission was 104°, the pulse 135 and respirations 40. The patient remained delirious, requiring restraint. His condition became progressively worse and death ensued on the second day after admission.

Autopsy 1905, 82, was performed seven hours post-mortem. Anatomical findings: Acute lobar pneumonia; acute pleuritis; acute splenitis; chronic pericarditis; congestion and oedema of lungs. Gross examination showed a well-marked consolidation of the left lower lobe. On section the lower two-thirds of the pneumonic lobe were found in the stage of gray hepatization. The upper third was moist, granular, and dark red in color. The pleura was bathed with a fibrino-purulent exudate. The left upper lobe showed congestion and oedema. The right lung was negative. Duplicate smear-preparations were made from all the lobes. One of each was stained by Gram's method and counter-stained with pyronin. The duplicates were stained for capsules. All of the lobes showed encapsulated diplococci. The smears from the pneumonic lobe showed comparatively few diplococci contrasted with the number from the normal lobes. In all the smears there were phagocytic cells which contained from two to sixteen pair of Gram-positive lanceolated diplococci. Still other phagocytic cells contained innumerable Gram-negative bacilli. Here again the greater numbers were observed in smears made from the uninvolved lobes.
A series of twenty-five plates was seeded, five plates of uniform seeding representing each lobe. All plates developed colonies of the pneumococcus, Streptococcus pyogenes, B. influenza, B. pseudo-diphtheriae, Staphylococcus albus and aureus. The number of colonies of pneumococcus and B. influenza was greater on the plates representing parts of the lung other than the pneumonic area. The colonies of B. influenza ranged from one to three hundred per plate. Though all the plates developed colonies of Staphylococcus aureus, no one plate contained more than fifty. The number of colonies of pseudodiphtheria bacillus was relatively small. Plates were also made from the heart’s blood (1 c.c. of blood being suspended in 10 c.c. of diluant). Here again developed pneumococcus, streptococcus, B. influenza, B. pseudo-diphtheriae, and staphylococcus. A few plates contained so many colonies of B. influenza that it was impossible to determine their number.

From the study of these two cases no conclusions are warranted.

II. Surgical Material.—The results of the examination of surgical material can be summarized briefly.

Pneumococcus present in pure cultures:
- Alveolar abscess....................... 1 case.
- Abscess in groin....................... 1 case.
- Abscess in chest....................... 1 case.
- Pelvic abscess......................... 1 case.
- Septic knee............................ 1 case.
- Septic uterus.......................... 2 cases.
- Acute mastoiditis....................... 2 cases.

Pneumococcus and streptococcus:
- Abscess of shoulder..................... 1 case.

The study of pleural exudates deserves special mention. Twenty-five examinations have been made.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumococcus alone</td>
<td>9</td>
</tr>
<tr>
<td>Pneumococcus and streptococcus</td>
<td>6</td>
</tr>
<tr>
<td>Streptococcus alone</td>
<td>10</td>
</tr>
</tbody>
</table>

It is noteworthy that in the early examinations it was considered sufficient to identify either the pneumococcus or the streptococcus, and no consistent effort was made at any time to grow both the pneumococcus and the streptococcus.

III. Cerebro-Spinal Fluid.—From the cerebro-spinal fluid taken by lumbar puncture from two cases, the pneumococcus was isolated in pure culture. The material was sent from outside the hospital and no subsequent history has been obtained.

IV. Sputum.—By the plate method the pneumococcus has been isolated from the sputum of several cases of pneumonia.
Examinations of sputa from cases of chronic bronchitis and suspected tuberculosis have shown the presence of the same organisms. The pneumococcus was isolated eight times from tubes inoculated for the routine examination for diphtheria bacilli.

Saliva from the mouths of twenty-four individuals in the laboratory and hospital was examined. These people had never had pneumonia and were in perfect general health at the time of examination, no account being taken of the slight chronic lesions of the pharynx with which most people in this climate are affected in the winter months. Pneumococci were easily isolated in every instance. In this series of examinations no consideration was given to cultures which did not ferment inulin.

Saliva was suspended in bouillon and plated as above described. Colonies were transferred to blood-agar tubes. Stains were made from the growth twenty-four hours old by Gram's method and usually by methods for identification of capsules. If the morphology and growth were characteristic, the cultures were at once tested in inulin bouillon and, as before stated, only those producing a marked acidity, shown by titration, have been regarded as pneumococci. As there is no evidence that the streptococcus ever ferments inulin, these points seem sufficiently conclusive for the present purpose. Inulin-splitting pneumococci we conclude are present during the winter season in the saliva of practically every person living under ordinary city conditions.

Our studies on the distribution of the pneumococcus have shown that it is to be found practically always at autopsy in the lungs of people dying with lobar pneumonia, and that it is found in the healthy as well as in the diseased portions of the lung. At times this micro-organism is found in the non-pneumonic lungs of persons dying from causes other than pneumonia. Very frequently the pneumococcus is found in purulent affections of the serous cavities and in acute abscesses of other parts. In the mouth and throat of persons in good general health it is found so often that its presence can have no pathological significance.

It is further evident that Streptococcus pyogenes and the
pneumococcus are found in much the same places and conditions and very often occur together. The facts of general distribution hardly warrant the supposition that the one bacterium has a closer etiological relation to acute lobar pneumonia than the other. A more extended and careful study of the local distribution of these two organisms in pneumonic lungs might, however, lead to valid negative or positive conclusions on this most important point.

CHARACTERISTICS OF THE PNEUMOCOCCUS IN RELATION TO ITS SOURCE OF ORIGIN.

In attempting to draw distinctions between pneumococci of various sources we have met with no success. We have paid most attention to cultural features. The fermentation of inulin characterizes a definite group of pneumococci. Inulin-splitting pneumococci are found with frequency among those cultivated from all sources. We have found no other cultural feature which is more distinctive.

We have also considered the virulence of cultures to some extent. We have tested the virulence of thirty-five recently isolated cultures, in large part from the pneumonic lung. We have used young rabbits. The dosage has been either 10 c.c. of a twenty-four-hour glucose-bouillon culture showing heavy growth or the whole of a twenty-four-hour culture on glucose agar plus rabbit's blood. In some instances two or three such agar cultures have been given. The injection has usually been made into the peritoneum. For control, animals have been injected subcutaneously and intravenously. In only one instance has a fatal result been attributable directly to the culture; the organism was isolated from the saliva of a healthy person. We do not consider our work on these points sufficiently extensive to be entirely conclusive; so far as we have gone, our cultures from all sources have been identical in possessing a low grade of virulence towards laboratory animals and in showing no cultural variation coincident with their source.

As bearing on the results of previous workers who have written
on the virulence of the pneumococcus, we offer the following observations. Depending on the plate method for isolation, we have frequently inoculated into animals the original material, namely, sputum, pus, or suspensions from the lung. The results of these inoculations fall into two distinct classes. In several instances the pneumococcus was present in pure culture as estimated by smear preparations and plate culture. In each of these cases the animals survived the injection of large quantities of material. One young rabbit received 5 c.c. of pus from the base of the brain in a case of pneumococcus meningitis. The examination of smear preparations showed large numbers of encapsulated pneumococci in a good state of preservation. Two of these animals still live. Two were killed on the fifth day and the pneumococcus was recovered from their blood.3

In the other class of cases the material examined contained a mixture of bacteria. The injection of small or moderate doses gave in most instances a fatal result in from one to four days. The pneumococcus was always found in the blood, generally in pure culture. If bacilli belonging to the Friedländer group were present, they were frequently found in the blood of the inoculated animal, especially when the guinea-pig was used. In view of the series of experiments with material containing the pneumococcus only, and the consistently negative results with pure cultures, we doubt whether the presence of the pneumococcus in the blood of these animals indicates that it alone is responsible for the death of the animal.

It seems probable that when the blood of the animal first inoculated is transferred directly to another animal, a false impression of the virulence of the culture finally recovered may be given, because of the exalted virulence attained by the direct passage from animal to animal.

THE ACTION OF PNEUMOCOCCI ON INULIN.

Early in the year we placed great reliance on the inulin test as an absolute means of differentiating the pneumococcus from

3 The culture isolated from the rabbit inoculated with pus from this case of meningitis is described in more detail in our discussion of the non-inulin-fermenting pneumococci.
Streptococcus pyogenes. We used then the serum-water-inulin medium proposed by Hiss. Several hundred tubes of the serum-water medium made with inulin ("Merck") and beef-serum gave consistent results with ten pneumococcus cultures and several cultures of Streptococcus pyogenes.

These cultures have been retained and have served as types with which our organisms isolated later have been compared. Except for the one lot of inulin-serum-water medium mentioned, which fortunately was large, we have had nothing but failure in its use. This failure we have been unable to explain satisfactorily. We do not feel convinced that the medium is essentially faulty, but there has been perhaps some disturbing factor in our material or method of preparation which we have not been able to discover or control. We have, however, had no trouble in obtaining constant results with the pneumococcus in serum-water media containing other carbohydrates.

In trying to explain the inconstancy of our results we have proceeded as follows: The reaction of the medium has been carefully controlled and varied from neutral to 1% acid to phenolphthalein with differences of .1% of acid. These differences bring about distinct variations in the opacity of the preparation. But when inulin is added no one degree of acidity is found to give more consistent results than another. Inulin has been sterilized separately and added to the tubes with a pipette, but the results have been no better. We have worked with the following preparations of inulin:

- Merck's inulin, white.
- " alant starch, Lot I.
- " " white, Lot II.
- " inulin ("highest purity").
- Bausch and Lomb's inulin, white.

The preparation which gave the one consistent result was Merck's, Lot II. Repeated trials with the same lot have failed. The two lots of Merck's white inulin and Bausch and Lomb's white inulin when tested in 10% solution in distilled water with Pehling's solution have shown reducing substances present in quantity equivalent to from 2 to 3% of dextrose in the dry
Studies on the Pneumococcus

If a 10% solution in distilled water is subjected to sterilization in the autoclave (at 10 lbs. pressure for ten minutes), the percentage of reducing substances may be increased to five. These reducing substances may not be dextrose and they may indicate the presence of other impurities which do not reduce the copper. One small quantity of inulin (Kiliani) obtained from Bausch and Lomb was subjected to the autoclave before the possibility of injury had suggested itself. Subsequently it was found to contain about the same percentage of the reducing substances as that shown by other preparations. This preparation we have not been able to buy again.

Inulin Bouillon.

In the hope of avoiding the difficulties encountered in the use of the inulin-serum-water medium, at the suggestion of Dr. Theobald Smith we undertook a series of experiments with inulin in bouillon. The outcome exceeded our expectations. The results of successive titrations remained uniform, and we can recommend this modification of the inulin test for routine work. Using these results as a basis, pneumococci can be classified as follows:

I. High acid producers.
   A. Inulin fermenters
   B. Inulin non-fermenters

II. Medium acid producers.

III. Low acid producers.

Bouillon is made according to standard methods from beef. It is reduced in acidity to from .2 to .4% normal to phenolphthalein. Subsequent sterilization restores the acidity to from .5 to .8%. Carefully cleaned tubes containing 9 c.c. of bouillon are sterilized. A 10% solution of inulin in distilled water is sterilized in the autoclave at 15 lbs. pressure for fifteen minutes, and with a sterile pipette 1 c.c. of the inulin solution is added to each tube of bouillon.

The tubes are simultaneously inoculated with the cultures to be tested, incubated for the period of the experiment, and then steamed for a short time in an Arnold sterilizer. They are then titrated for percentage acidity, using the same solution and in-
indicator. Theoretically it might be better to prepare as a culture base sugar-free bouillon. It has proved satisfactory, however, to use as control cultures in bouillon containing no inulin.

The table on page 486 shows the result of titrations in two lots of inulin-bouillon made from different material and different brands of inulin. The period of growth in this experiment is one week. With a little experience the high and medium acid-producing organisms can be distinguished in twenty-four hours, and the low acid-producing organisms in forty-eight hours, although the numerical differences at these periods are small and inconstant. It is probable that if the number of cultures worked with were doubled or tripled, organisms intermediate between these groups and types might be found, but the types are sufficiently marked to illustrate the efficiency of the method employed. With two or three exceptions, all of our inulin-fermenting organisms that have survived prolonged cultivation appear in the table. The non-inulin-fermenting group might be greatly enlarged by the addition of about thirty cultures which we still retain. As the differentiation of many of these cultures from Streptococcus pyogenes is difficult and uncertain, we prefer to test only those whose identity rests on secure ground.

The classification is based on the reactions of cultures which have been several weeks under cultivation. We have recently made a number of titrations with freshly isolated cultures. Many of them have given reactions of from 5.8 to 6.1 % of a normal acid solution at the end of a week. We should hesitate, therefore, to separate the two cultures of Type I from those of Type II on this basis alone. As they are the only cultures among our stock which ferment mannit, and as their titration is always somewhat higher than that of any other culture, we feel that the distinction is justified. The cultures of Group A we think are all pneumococci.

Group B requires explanation. By comparison of the figures given for the plain bouillon controls with those given for the members of Type IV, it is seen that the average of the latter is somewhat higher. This is probably due to the presence of small quantities of fermentable impurities in the inulin. Inspection
Studies on the Pneumococcus

GROUP A = Inulin Fermented.

<table>
<thead>
<tr>
<th>Type</th>
<th>Culture Number</th>
<th>Total Acidity Bouillon.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I C.D. 16</td>
<td>1.1%</td>
<td>6.5%</td>
<td>6.8%</td>
</tr>
<tr>
<td></td>
<td>C.D. 17</td>
<td>1.0%</td>
<td>6.6%</td>
</tr>
<tr>
<td>II C.D. 9</td>
<td>0.9%</td>
<td>4.3%</td>
<td>4.3%</td>
</tr>
<tr>
<td></td>
<td>C.D. 10</td>
<td>1.0%</td>
<td>4.3%</td>
</tr>
<tr>
<td></td>
<td>C.D. 11</td>
<td>1.1%</td>
<td>4.5%</td>
</tr>
<tr>
<td></td>
<td>C.D. 12</td>
<td>1.1%</td>
<td>4.8%</td>
</tr>
<tr>
<td>III C.D. 1</td>
<td>1.0%</td>
<td>2.6%</td>
<td>2.6%</td>
</tr>
<tr>
<td></td>
<td>C.D. 2</td>
<td>1.1%</td>
<td>2.3%</td>
</tr>
<tr>
<td></td>
<td>C.D. 3</td>
<td>1.1%</td>
<td>2.8%</td>
</tr>
<tr>
<td></td>
<td>C.D. 4</td>
<td>1.2%</td>
<td>2.6%</td>
</tr>
<tr>
<td></td>
<td>C.D. 5</td>
<td>1.2%</td>
<td>3.3%</td>
</tr>
<tr>
<td></td>
<td>C.D. 6</td>
<td>1.1%</td>
<td>3.3%</td>
</tr>
<tr>
<td></td>
<td>C.D. 7</td>
<td>1.1%</td>
<td>3.0%</td>
</tr>
<tr>
<td>IV C.D. 7</td>
<td>0.9%</td>
<td>1.4%</td>
<td>1.5%</td>
</tr>
<tr>
<td></td>
<td>C.D. 27</td>
<td>8.8%</td>
<td>1.6%</td>
</tr>
<tr>
<td></td>
<td>C.D. 14</td>
<td>1.0%</td>
<td>1.1%</td>
</tr>
<tr>
<td></td>
<td>C.D. 28</td>
<td>1.1%</td>
<td>1.6%</td>
</tr>
<tr>
<td></td>
<td>C.D. 29</td>
<td>0.9%</td>
<td>1.7%</td>
</tr>
<tr>
<td></td>
<td>C.D. 30</td>
<td>1.1%</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

Note: Streptococcus pyogenes cultures titrate up to 1.6% acid.
of the titrations with this group shows that the method gives no aid in the identification of these cultures. The cultures included in the table, judged by all of their characters, are without doubt pneumococci. We believe, however, that some cultures which according to the titration with inulin bouillon belong in this group are typical streptococci. It is not our purpose at present to classify the cultures of this group. In order to make clear our point, that there are pneumococci which do not ferment inulin, we give the following description and history of such a culture.

Culture C. D. 7.—Isolated by plate method in December, 1904, from the lung of a case of acute lobar pneumonia. A large number of colonies were examined, but no inulin fermenter was found. C. D. 7 was at that time a Gram-positive diplococcus. The lanceolate pairs were surrounded by a large capsule which could be easily stained by either of the Hiss methods. The capsule persisted for some weeks in culture, but was finally lost. The colonies on Loeffler’s blood-serum and rabbit’s blood-agar were large and moist. Growth in glucose-bouillon was profuse, the medium being diffusely clouded. Litmus milk was coagulated in twenty-four hours, and in forty-eight hours the litmus in the lower half of the tube was decolorized. Lactose-serum water was coagulated in twenty-four hours.

At the present time (June 20, 1905) these characters, excepting the capsule, are well preserved. Glucose-bouillon is still diffusely clouded, and a Gram stain made from a bouillon tube shows a predominant number of rather asymmetrical pairs of large lanceolate cocci. There are many chains, but they are all short, containing from four to ten cocci. The morphological characteristics are intact, and as well marked as at the time of isolation. This culture has never coagulated the serum-water medium, and in our titrations with inulin-bouillon its total acidity has never gone above 1.5 % acid.

Although we have given to few cultures among the non-fermenting group so close attention as we have to C. D. 7, we are certain that others of these cultures are pneumococci and that in routine examinations the non-inulin-fermenting group will be frequently encountered. Since the introduction of inulin has made the identification of some groups of pneumococci relatively easy, the difficulties previously encountered in distinguishing this organism from Streptococcus pyogenes are significant only in the non-inulin-fermenting group. The cultures which ferment inulin, and a few of those which do not, have the pneumococcus characters so well developed and preserve
them so constantly in culture that their identification by means formerly available is not very difficult. On the other hand, it is probable that careful study of the non-fermenting group will establish intermediary varieties and may render unavoidable the conclusion that the pneumococcus and the streptococcus are races of a single species of bacterium. That the pneumococcus and streptococcus are not the extreme types of such a species seems not improbable if one takes into consideration the saprophytic streptococcus on the one hand and such cultures as the encapsulated diplococcus of which we will give an account on the other. There may also be varieties of the species which have been separated even farther from the usual types. For example, we will give in detail the record of a culture previously mentioned:

_Culture from case of cerebro-spinal meningitis_ (Autopsy 1905, 33).—Stained preparations from the pus at the base of brain showed large numbers of encapsulated diplococci staining by Gram’s method. Cultures on Loeffler’s blood-serum and blood-agar were negative so far as micro-organisms other than pneumococci were concerned. There was no growth on the surface, but careful examination of the condensation water showed a few encapsulated Gram-staining diplococci. Subcultures were negative. Five cubic centimeters of the pus at the base of the brain were inoculated into the peritoneal cavity and subcutaneous tissues of a rabbit weighing 750 grams. After twenty-four hours, the animal ate well and had lost no weight. On the fifth day it was killed. Examination of the heart’s blood showed a few lanceolate diplococci retaining Gram’s stain and possessing a distinct capsule. Cultures on Loeffler’s blood-serum were negative. In the condensation water of blood-agar tubes, a few diplococci agreeing in negative and positive character with those from the original material were found. After repeated and frequent subcultures covering a period of six weeks, during which time proliferation was very slight and confined to the condensation water, the diplococcus rather suddenly increased its activity of growth. It grew fairly well in fine colonies on the surface of blood-agar, but did not grow on plain agar or on Loeffler’s blood-serum. In milk it produced a slight acidity after several days. No fermentation of sugars could be demonstrated in the serum-water-media. After two or three generations of this relatively abundant growth its activity again declined, and in spite of repeated efforts the culture died out. Cultivation failed though the reaction and sugar content of media were varied and blood and serum were added.

The organism just described was evidently a pneumococcus which differed very widely from the members of this group usually encountered. It had morphological characteristics which,
as shown in Fig. 5, were sufficiently definite to classify it as pneumococcus. Perhaps one could conclude from the experience with this culture that there are parasitic pneumococci so delicate as to be unrecognized by our present methods of cultivation and at the same time possessing possibly pathological significance.

It might be said that the method which we propose for using inulin is too tedious for routine work. To obviate this difficulty, we have made control observations with inulin-bouillon made in bulk and sterilized after tubing. The quantity in each tube need not be the same, and the medium is as efficient as that more carefully made. The inulin when sterilized in the bouillon does not undergo sufficient reduction to injure the efficiency of the test. The time we have used for the reported tests (one week) is necessary to bring out the details of grouping which we have described, but, as has already been mentioned, organisms belonging to different groups can be roughly separated in a shorter time. Neither is the titration a necessity; the medium and high acid-producing subdivisions cause in the inulin-bouillon a diffuse cloud and heavy sediment which are apparent in from sixteen to twenty-four hours. The low acid-producers show a well-marked diffuse cloud in the medium on the second day. The non-fermenters never cloud the bouillon and the sediment of bacterial growth remains slight. Though titration is not necessary for the recognition of the fermenting pneumococci, it has served to distinguish finer differences between groups, and as a routine optical method the bouillon has all the advantages of the serum-water medium. We think that the group of low acid-producers cause coagulation irregularly, because the total acidity which they produce under unfavorable conditions is often just sufficient to change litmus, and this degree of acidity seems to approximate the coagulation point of the serum-water. The inulin-bouillon has the added advantage that its constituents are easily procured and combined, its constitution is more uniform, and the reaction produced is more delicate when estimated by titration for percentage of acidity than is the case with the serum-water medium.
We have retested the samples of inulin before used, and find that when used in the bouillon the brands labeled "Highest purity," which are hard to secure, are not essential. All the samples of white inulin mentioned before and one lot from another supply house act equally well.

The irregularity of the results obtained by us with inulin-serum-water medium has not been satisfactorily explained. Since the difference in favor of the bouillon might be referred to a greater quantitative development of bacteria in this medium, we have tested media with three actively-growing type cultures of the pneumococcus, varying in their fermentative activity, and with one culture of Streptococcus pyogenes. We have estimated by plating after twenty-four hours of growth the number of bacteria in plain bouillon, plain serum-water, dextrose-bouillon, dextrose-serum-water, inulin-bouillon, and inulin-serum-water. There was no difference in the number of colonies developing on the plates from the bouillon media and those from the serum-water media.

Our conclusions with regard to the inulin test are as follows:

I. The routine isolation of the pneumococcus is much facilitated by this test. Until the number and pathological significance of the non-inulin-fermenting group of pneumococci is established, the test must be carefully controlled by the older methods of identification.

II. A final opinion as to the best method of using the inulin cannot be offered. We believe, however, that the inulin-bouillon method by mere inspection is as easy to carry out and as accurate as the serum-water method, and that with careful titrations it is more delicate as it offers a means of subdividing the inulin-fermenting pneumococci into definite groups—a differentiation impossible by the latter.

**STREPTOCOCCUS MUCOSUS.**

In the course of the investigation we have twice encountered a Gram-staining, encapsulated diplococcus with characteristics which make it peculiarly interesting. We have identified it with the diplococcus described by Richardson, and by comparison
of cultures with the coccus described by Howard and Perkins. As we have noted some points in its morphology, cultural features, and pathogenicity which do not appear in their descriptions, we will describe the organism in some detail.

**Source.**—The first of our cultures of this micrococcus was isolated from the lung of a case of acute lobar pneumonia. The lung showed gray hepatization. Our description is based on the study of this culture. Recently a second organism which has the same cultural features has been obtained from the pus from a mastoid abscess.

**Morphology.**—Morphologically this micro-organism is an encapsulated, lanceolate diplococcus. It retains Gram's stain. Its capsule persists in cultures and is readily stained by any capsule method; it is frequently stained by the ordinary staining methods. Fig. 1, for example, shows a stained preparation made according to Gram from a culture on blood-agar. In the preparation, capsules were not noticed, but the photograph shows them faintly stained. Figs. 2, 3, and 4 are from preparations also stained by the method of Gram slightly modified. Smear preparations on clean slides are made from the exudate of an animal recently dead after inoculation with the culture. After drying in the air they are thoroughly fixed in the flame. While the slide is still hot enough to cause the stain to steam, the preparation is covered with the solution of gentian-violet, which is left on the slide for one or two minutes. Iodin is applied in the usual way, but decolorization with alcohol is not completed. The thin parts of the smear are completely washed out, the thicker parts are left stained. Counterstaining is omitted. Some fields will now be found in which there is a sharp differentiation of the cocci and in which the capsule as well has retained the blue. The results are inconstant and it may be necessary to make many preparations before a good one is obtained. The result when successful is a sharp picture in which the details of the capsule and its relation to the coccus are better shown than by other methods. By the study of these preparations, appearances which we have endeavored to show by photograph can be made out. Fig. 2 (a) shows a single coccus surrounded by a complete circular capsule.
Studies on the Pneumococcus

Fig. 2 (b) shows a pair of small cocci with a poorly defined line of demarcation between them. On opposite sides of the capsule and in the line of the transverse division between the individuals of the pair are a slight thickening of the capsule and a thin linear projection toward the center of the pair. At d in Fig. 2 and at c in Fig. 3 these lateral thickenings have increased in breadth and inward extension. The dividing line between the cocci is better marked. At e, Fig. 4, the division between cocci is complete and the capsule surrounds both elements.

The capsules can also be stained by other methods. If Wright's modification of the Romanowsky stain be used, the cocci take a blue stain and the capsular portion takes the pink color. The outline of the capsule is distinct and the points that we have endeavored to illustrate are brought out with greater delicacy.

Thionin blue in saturated aqueous solution stains the cocci and capsule from an exudate rather lightly. The stain must be steamed for a few moments and is then washed off with water. The capsule is delicately outlined in purple and the details before described are seen. The preparations made by these later methods are less suitable for illustration than those by Gram's method.

The capsules stain by either of the methods of Hiss, but the details here described are rarely brought out by them, the capsule appearing filled with the stain rather than outlined by it.

The capsule of this micrococcus is always present when the organism is growing and moist. It can be readily demonstrated by any of the special methods of capsule staining and with almost equal readiness by methods which do not ordinarily demonstrate capsules on the pneumococcus and streptococcus. In its development and division the capsule follows closely the division and separation of the coccus. By the modified Romanowsky stain the basophilic coccus can be well differentiated from the oxyphilic capsule. The capsule of this diplococcus is evidently more highly developed and differentiated than that of the pneu-
mococcus. In these facts there is some evidence that the capsule of this micrococcus may be a part of the bacterial cell. Possibly it is the precursor of the cytoplasm of the more highly developed unicellular plants.

_Cultural Features._—The micrococcus grows well with a profuse, very watery growth on the surface of 1% glucose-agar, .5% acid to phenolphthalein, if about 1% of “fresh” defibrinated blood be added. The individual colonies are perfectly transparent and attain in forty-eight hours a size 6 mm. in diameter. It also grows in a glucose-agar stab culture without blood if the medium is made from beef and is suitable in reaction. In this way there can be developed peculiar and, so far as our experience goes, characteristic wing-like lateral out-growths. This feature was noted by Richardson, and together with the persistence of the capsules in culture serves to identify his culture with those that we have isolated. In gelatin-agar mixtures of proper reaction and sugar content growth is good, but lateral outgrowths in stab cultures do not appear. Abundant growth can be obtained on any of the usual solid or fluid media if .5 c.c. of fresh blood be added to each tube. The published data on the fermentation reactions are scanty. Hiss states that the organism of Howard and Perkins ferments inulin. We find that if fresh defibrinated blood be added to tubes of serum-water medium containing dextrose, lactose, maltose, galactose, mannit, dextrin, or inulin, these organisms coagulate the medium in from twenty-four to forty-eight hours. Controls of plain serum-water plus blood remain fluid. In litmus milk our organism produces very slight acidity after some days. In this respect it seems to differ slightly from those as described by others, which become markedly acid in twenty-four hours.

We have compared our organism with that of Howard and Perkins, obtained through the kindness of Dr. Libman, of Mt. Sinai Hospital, New York. In morphology and character of growth on blood-agar and in dextrose-agar they are identical. They react in the serum-water-sugar media in much the same way. Both coagulate the sugar-containing media before mentioned, but there are slight differences in the reaction time. To
Studies on the Pneumococcus

Studies on the Pneumococcus

compare the acid production we have added 0.5 c.c. of fresh sterile defibrinated horse's blood to tubes containing 10 c.c. of bouillon with 1% of the various sugars. The results are shown in the following table:

<table>
<thead>
<tr>
<th>Acidity in Percentage of Normal Acid Solution.</th>
<th>Our Organism:</th>
<th>Organism of Howard and Perkins.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bouillon plus blood ................................</td>
<td>1.1%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Bouillon plus blood with 1% mannit .............</td>
<td>1.6%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Bouillon plus blood with 1% inulin .............</td>
<td>1.0%</td>
<td>3.4%</td>
</tr>
<tr>
<td>Bouillon plus blood with 1% dextrose ...........</td>
<td>4.0%</td>
<td>4.0%</td>
</tr>
<tr>
<td>Bouillon plus blood with 1% lactose ............</td>
<td>3.9%</td>
<td>3.8%</td>
</tr>
<tr>
<td>Bouillon plus blood with 1% maltose ............</td>
<td>4.0%</td>
<td>3.8%</td>
</tr>
<tr>
<td>Bouillon plus blood with 1% galactose ..........</td>
<td>3.9%</td>
<td>4.0%</td>
</tr>
<tr>
<td>Bouillon plus blood with 1% saccharose ..........</td>
<td>4.1%</td>
<td>3.8%</td>
</tr>
<tr>
<td>Bouillon plus blood with 1% dextrin ............</td>
<td>4.0%</td>
<td>3.3%</td>
</tr>
</tbody>
</table>

Uninoculated control .9 %

The figures show that our organism differs from that of Howard and Perkins in the amount of acid produced in the presence of inulin under given conditions. Neither of them produces marked acidity in the mannit-blood-bouillon. Since the serum-water medium with the addition of blood and each of these sugars was coagulated, the greater delicacy of the titration method of demonstrating these reactions is again shown. When the total acidity produced by a culture under the conditions of the experiment rests at about 1.5% normal acid, the reaction in the serum-water medium is apt to be uncertain. The identity of the reaction in serum-water with that in bouillon is again brought in question.

The hemolytic activity of these two cultures has been roughly estimated in the following way: to tubes of plain beef bouillon .5 c.c. of defibrinated horse-blood are added. Tubes are inoculated and incubated with controls. In twenty-four hours the height to which the color ring of dissolved hemoglobin rises in the
Charles W. Duval and Paul A. Lewis

The tube is noted. The hæmolysis is markedly greater with our organism than with that of Howard and Perkins. This seems to agree with the fact that when our culture was first isolated we had great difficulty in growing it except in the presence of blood. No such difficulty is noted by others who have isolated the organism. Efforts to obtain a hæmolysin or toxin that would pass a filter have so far been without result.

Pathogenicity.—The diplococcus with which we have worked is pathogenic for mice, guinea-pigs, and rabbits. When first isolated, one loop of blood-agar culture twenty-four hours old sufficed to kill a guinea-pig or young rabbit in twenty-four hours with general septicemia and local exudation. At present several loops are necessary to bring about the same result. Smaller doses cause death in several days with fibrinous inflammations of the serous cavities. The exudate is characteristic; with some exceptions the amount of fibrin is small, but the total exudate is relatively large. It is white, and has the consistency of thick mucilage. If dropped from a pipette into salt solution, the drop holds together, sinks to the bottom, and remains intact for some time. There is little tendency to hæmorrhage either after subcutaneous or intraperitoneal inoculation. One old rabbit which survived a small dose developed after several weeks multiple arthritis with much exudate in the joint cavities.

Previous observers have considered this organism a streptococcus. The names Streptococcus capsulatus and Streptococcus mucosus have been applied to it. Morphologically it seems much more closely related to the pneumococcus. The lanceolate diplococcus form and arrangement are well marked, as shown by Fig. 1, and are well preserved under prolonged cultivation. Chains occur, but they are no larger than is usual with the pneumococcus. They seem to occur under much the same unfavorable conditions that tend to chain formation with the pneumococcus, and may possibly represent an involution. In its fermentation reactions it is very active and more closely resembles the pneumococcus than the streptococcus, although our culture differs from that of Howard and Perkins in this respect. Its pathological reactions also resemble the pneumococcus in that the organism appears in
the blood after injection of pure or mixed culture somewhat more readily than does the streptococcus. The organism is apparently a highly specialized pneumococcus.

DESCRIPTION OF PLATE.

Fig. 1.—Streptococcus mucosus. Stained by Gram's method and magnified 1000 diameters to show the morphology of the culture described.

Figs. 2, 3, 4.—Streptococcus mucosus. Stained by the modified Gram's method and magnified 1000 diameters to show capsules.
   a. Single coccus with complete capsule.
   b. Capsule shows slight lateral thickening and line projecting toward center.
   c. Very heavy lateral thickenings in capsule.
   d. Somewhat heavier lateral thickenings in the capsule.
   e. Capsule completely surrounding two cocci.

Fig. 5.—Pneumococcus from a culture on blood-agar twenty-four hours old. Stained by Hiss' copper-sulphate method for capsules and magnified 1000 diameters. No serum was added to the preparation.

Fig. 6.—Culture of pneumococcus (C. D. 30) on blood-agar twenty-four hours old (second generation); stained by Gram's method and magnified 1000 diameters. Showing the formation of chains composed of rather distinct lanceolate pairs.