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STUDIES IN INFLUENZA AND PNEUMONIA

STUDY V. OBSERVATIONS ON THE BACTERIOLOGY AND CERTAIN CLINICAL FEATURES OF INFLUENZA AND INFLUENZAL PNEUMONIA

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The bacteriologic studies of the epidemic of influenza of 1889-1890,[13] of lesser outbreaks prior to the pandemic of 1918,[15] and the preliminary studies of the pandemic by others have shown that while influenza bacilli occur commonly the organisms of the pneumococcus-streptococcus group are constantly associated with this disease. In the course of my studies on this group of organisms and the diseases due to them I have been impressed repeatedly by the marked changes they undergo at times, particularly in infecting powers and immunologic reactions. In some instances these changes appeared to be true mutations.[25] It was thought possible that the peculiar picture presented in influenza, such as the marked prostration, cyanosis, leukopenia, and its almost simultaneous appearance over wide areas might be due to variants or mutation forms of organisms commonly present in the respiratory tract of man. Accordingly, as the epidemic reached Rochester a comprehensive plan of study, taking into consideration this possibility, was determined on. The results obtained form the basis of the series of experiments that I report. Preliminary statements, including a description of the somewhat peculiar green-producing streptococcus isolated quite constantly,[22] of immunologic studies,[24] and of the extraordinary invasive power of the streptococci from influenza on intratracheal application,[23] have been published.

In this paper are recorded the more important bacteriologic findings obtained throughout the four epidemic waves of influenza which occurred in Rochester during the autumn and winter of 1918-1919, and these findings correlated with certain clinical features of the disease.

The epidemic of 1918 began in Rochester during the latter part of September. An emergency hospital was opened and when this became inadequate certain parts of other hospitals were set aside for the care of influenza patients. The source of the material studied was, in the main, from patients admitted to these hospitals, most of whom had

come to the Mayo Clinic for the treatment of some other condition or to accompany patients. A large number of cultures, however, were made from persons residing permanently in Rochester who had contracted influenza. A large proportion of the first group came long distances from widely separated communities, and many developed symptoms before or soon after their arrival. Some no doubt were infected en route or before leaving home; hence, the cases studied represent a heterogeneous group and the findings accordingly may be regarded as quite representative of the epidemic.

TECHNIC

Cultures were made from throat swabs prepared in the usual manner, from sputum, anterior nares, lung exudate, trachea and bronchi, peritracheal lymph gland, pleural fluid, the blood after death, and in some instances, from the blood during life. The sputum was collected in sterile, wide-mouthed glass vials and taken to the laboratory while fresh. Cultures were made on the surface of blood-agar plates directly or after washing in sodium chlorid solution, and into tall columns of dextrose broth, dextrose-brain broth or dextrose-blood broth. Owing to the mucoid and serous character of the sputum in influenza washing the sputum was quite unsatisfactory since it led to too great a dilution and hence the plates were made routinely by spreading a rather large amount of the unwashed sputum (about 0.1 c c) directly over the plates by means of triangular-shaped spreaders made from flat nicrome wire. The material was spread over the whole surface of the plate, part of the plate was heavily, and part lightly inoculated. The plates were incubated twenty-four hours at from 33 to 35 C. and then read. If influenza bacilli were not present the plates were incubated for an additional twenty-four or forty-eight hours. The air in the incubator was kept saturated with moisture by means of an open dish of water and by reducing the amount of ventilation to the minimum. The results of the cultures on the plates were recorded according to the numerical scale of 1 to 4; 1 indicating from 1 to 10 colonies; 2 from 11 to 100 colonies; 3 from 101 to 1,000 colonies and 4, 1,000 colonies and above, of the different bacteria. After death material was collected in sterile pipets by the pathologist in charge and brought directly to the laboratory for examination. The cultures of this material were made in the same manner as those from the sputum. Smears from throat and sputum during life, and of material after death were made,

stained for bacteria and examined, in some cases in order to check the results of the cultures, and to study the proportion and character of the cells and their behavior toward the bacteria present. In order to check the results of cultures and direct examination of the exudate still further, and to determine which of the bacteria nearly always present had the greatest invasive powers in influenza, the sputum and exudates of some of the cases were injected intraperitoneally and intratracheally into guinea-pigs and intraperitoneally into white mice. Smears and cultures of the peritoneal or lung exudate and blood of these animals were made according to the method just described.

At first plain agar, made from beef extract and peptone, to which 5 per cent. of defibrinated human blood was added, was used, but since influenza bacilli were not detected on these plates, the so-called "hormone" or "vitamine" agar was substituted. The medium was carefully titrated to + 0.6 per cent. to phenolphthalein, and cleared by the use of a centrifugal machine. Usually, however, it was slightly opalescent from fat which was not wholly removed from the meat. One and seven-tenths per cent. agar was added instead of from 2 to 2.5 per cent. as is usually done. To this medium, cooled to about 60 C. approximately 4 per cent. of defibrinated human blood was added before it was poured into the plate. The plates were not usually incubated previously so that the surface of the agar was not hard and dry, but soft and moist. The advantages of this medium over the plain agar medium for growing influenza bacilli were striking. Large numbers of typical influenza bacillus colonies developed from the sputum of patients when few or none had been detected previously on plain-blood agar. In order to make sure that the lack of growth of bacilli on the plain blood-agar used previously was not due to their absence, but to a difference in the medium, parallel cultures on the two mediums were made of the sputum of 8 cases of typical influenza occurring at the outset of the first wave. All hormone-blood-agar plates showed countless numbers of influenza bacilli which grew in symbiosis with the green-producing streptococcus or pneumococcus, hemolytic streptococcus, and staphylococcus colonies, whereas the plain blood-agar plates showed few or no colonies of influenza bacilli. The colonies were usually found in the heavily inoculated part of the plate and surrounding colonies of green-producing streptococci and staphylococci, but were found also in other parts of the plate. Cultures from throats of some of these patients yielded similar results although influenza bacilli were present in larger numbers. Because of these findings, this medium was adopted for

routine platings throughout the study. During the latter part of the work the special mediums for the isolation of influenza bacilli such as Avery's oleate agar [1] and of "chocolate" blood-agar plates were also used.

RESULTS

Cultures were made of the sputum or from the exudate of the throat of 571 patients with influenza or influenzal pneumonia during life, of the lung exudate, peribronchial lymph glands, or the blood after death in 107 cases. In 309 of the group of 571 and in 65 of the 107, both the clinical history and pathologic findings left no doubt as to the diagnosis. In the remaining 262 cases complete histories were not available for final analysis, but the diagnosis of influenza or influenzal pneumonia was made by the physician in charge at the time of the attack. The findings after death in 42 of these were clearly those of influenza, and since the bacteriologic findings in all were similar to those in the undoubted cases the diagnosis of influenza may be considered quite accurate.

TABLE 1

RESULT OF CULTURES OF MATERIAL FROM INFLUENZA AND INFLUENZAL PNEUMONIA

Bacteria	Material Cultured, Blood-Agar Plates					
	Sputum During Life (571 Cases)			Lung Exudate after Death (107 Cases)		
	Predominating or in Pure Culture, Per-centage	Not in Predominating Numbers, Per-centage	Total Per-centage	Predominating or in Pure Culture, Per-centage	Not in Predominating Numbers Per-centage	Total Per-centage
Green-producing streptococci or pneumococci	41	54	95	18	37	55
Hemolytic streptococci	9	29	38	23	54	77
Staphylococci	17	55	72	10	40	50
Bacillus influenzae	5	8	13	0	5	5
Micrococcus catarrhalis	0	6	6	0	3	3
Bacillus mucosus	0.8	1	1.8	4	7	11
Bacillus coli	0	0.8	0.8	3	15	18

The results obtained are summarized in Table 1. The figures giving the number of instances in which the various bacteria were found are omitted from the table and only those indicating the percentage are given, thus making direct comparisons readily possible. Fractions of a per cent. below 0.5 are dropped, to those 0.5 or above, one is added. The tabulations shown represent cases and not specimens. In many cases the sputum was cultured repeatedly during the course of the initial influenzal attack and during the influenzal pneumonia which

followed. The figures in the first and fourth columns indicate the percentage incidence in which the different bacteria were present in predominating numbers or in pure culture; in the second and fifth columns the percentage incidence in which the bacteria were present, but not in predominating numbers; and in the third and sixth columns the total percentage incidence to the occurrence of the different bacteria in the sputum and lung exudate respectively.

Green-producing streptococci (some of which fermented inulin) were isolated from the sputum in predominating numbers or in pure culture in 41 per cent., and not in predominating numbers in 54 per cent., a total of 96 per cent. of the 571 cases studied; they were isolated from the lung exudate after death in predominating numbers or in pure culture in 18 per cent., and in smaller numbers in 37 per cent., a total of 55 per cent. of the 107 cases in which necropsies were made. Hemolytic streptococci occurred in the sputum in predominating numbers or in pure culture in 9 per cent., and not in predominating numbers in 29 per cent., a total of 38 per cent. of the cases studied during life; they occurred in the lung exudate in predominating numbers or in pure culture in 23 per cent., and not in predominating numbers in 54 per cent., a total of 77 per cent. of the cases after death. Staphylococci were isolated in varying numbers from the sputum in 72 per cent. and from the lung exudate in 50 per cent. of the cases. The influenza bacillus was isolated from the sputum in 13 per cent., and in predominating numbers in 5 per cent.; it was isolated from the lung exudate in 5 per cent. of the cases, never in predominating numbers. Micrococcus catarrhalis was found in the sputum in 6 per cent., and in the lung exudate in 3 per cent. of the cases, always in small numbers. The Bacillus mucosus was found in the sputum in 1.8 per cent., and in the lung exudate in 11 per cent. The colon bacillus occurred in the sputum in 0.8 per cent., and in the lung exudate in 18 per cent. of the cases.

The figures represent only in a general way the relative importance of a series of different types of bacteria isolated during life and after death. Thus the incidence of staphylococcus in predominating numbers in the sputum in 17 per cent. of the cases should not be taken to mean that this organism was the cause of the attack in this percentage of cases, because in many only one or two cultures were made, often late in the disease when staphylococci had become relatively numerous. The lowering in total incidence of green-producing streptococci of from 96 per cent. in the sputum to 55 per cent. in the lung exudate, and the increase in incidence of hemolytic streptococci of from 38 per

cent. in the sputum to 77 per cent. in the lung exudate may be regarded as expressing roughly the importance of these two types of streptococci as causes of death in influenzal infection. Instances occurred in which the bloody fluid from the lungs of patients who died from acute hemorrhagic edema showed large numbers of green-producing streptococci or hemolytic streptococci in pure culture, as well as mixtures of these two types of streptococci. Cultures made from throats of patients who could not raise sputum at the onset of the attack and in some as controls of sputum cultures, showed similar results to those obtained from the sputum. Throat cultures at the beginning of influenza often showed large numbers of the green-producing colonies, in pure or almost pure form.

The number of colonies of the different bacteria which developed on the blood-agar plates was recorded according to the numerical scale of 1 to 4. It was thought worth while to determine the figure expressing the average incidence by days of the three main varieties of bacteria (green-producing streptococci, hemolytic streptococci, and staphylococci) which were isolated in influenza and in influenzal pneumonia throughout the four epidemic waves. This was done by adding the figures representing the number of colonies of each organism and dividing this sum by the total number of specimens of sputum cultivated on the different days. There was no noteworthy difference in the average incidence of the various bacteria for influenza and for the first five days of influenzal pneumonia; hence these cases are considered together. The number of green-producing streptococci averaged highest each day for the first five days, the average figures being 3.7, 3.4, 3.6, 3.6 and 3.4, respectively. The average figures for the first five days for staphylococci were 2.0, 2.4, 2.2, 3.0 and 2.4. The average figures for hemolytic streptococci were 1.2, 1.6, 1.8, 1.7, and 2. In numbers staphylococci occupied the middle position and hemolytic streptococci the lowest position, but there was a tendency to an average increase in staphylococci and hemolytic streptococci during the later stages of the disease. The figures representing the average incidence of the different bacteria after the fifth day varied between wide limits. Thus the figures expressing the average increase of green-producing streptococci occupied the middle position on the sixth, seventh, eighth, tenth, and eleventh days; hemolytic streptococci, the lowest position on the sixth, seventh, eighth, tenth and eleventh days, the highest position on the ninth and twelfth days; staphylococci, the highest position on

the sixth, seventh, eighth, tenth, and eleventh days, and the middle position on the ninth day. It becomes apparent, therefore, that the time in the attack when the cultures are made must be considered in order properly to interpret their meaning.

Marked variations in the type of bacterial flora were often noted at different stages of the disease during life and after death, and at different periods in the epidemic waves. In almost all instances of undoubted cases of influenza green-producing streptococci, together with a variable number of staphylococci or Micrococcus catarrhalis were the predominating flora at the outset of the influenzal attack throughout the epidemic waves. In most of the patients who recovered without developing pneumonia and in many who developed nonfatal or fatal attacks of pneumonia during the earlier part of the waves, this flora persisted. Later in the epidemic waves, however, there was a tendency for the green-producing streptococci to be displaced by hemolytic streptococci. This tendency to an increase in hemolytic streptococci in the later stages of the disease was noted especially in the patients who succumbed to the infection. Thus, in 21 fatal cases in which repeated cultures were made of the sputum during life and in which the lung exudate was cultured after death, green-producing streptococci predominated in the sputum during life in 16, and hemolytic streptococci in 5, while in these same cases hemolytic streptococci predominated in 11, and green-producing streptococci in 10, in the lung exudate after death. There was a shifting, therefore, from a predominant green-producing streptococcal flora to a hemolytic streptococcal flora in 6 of these fatal cases. The sputum usually became bloody as this occurred; the blood count showed no noteworthy change. The interval from the time of the sputum cultures to the time of the lung culture in these 6 cases was 1, 2, 4, 12, 12, and 4 days, respectively, or an average of 5.8 days. In some of the patients who recovered, on the other hand, the green-producing streptococcal flora again became predominant as the symptoms disappeared. This shifting of bacterial flora occurred both in cases of influenza without demonstrable lesions in the lung, as well as in influenzal pneumonia, but it occurred more often in the latter condition. The lack of agreement in the flora was noted not infrequently in cultures from the blood, from the lung, and from the pleural and other exudates after death. At times this occurred simultaneously in groups of patients who contracted the disease at about the same time. The blood after death proved to be sterile in about one-third of the cases cultured, and in the others green-

producing streptococci or hemolytic streptococci alone or with staphylococci were isolated in about an equal number of cases. Green-producing streptococci were, at times, found in pure culture in the blood when the lung and other exudates showed few or no green-producing streptococci, but hemolytic streptococci with or without staphylococci. The cases which showed empyema usually yielded a predominating number of hemolytic streptococci.

The lack of agreement in the type of streptococcus colonies isolated from material after death, as noted in some cases, is well illustrated in the cultures from a case of fulminating influenzal bronchopneumonia and ulcerative laryngitis. The necropsy was performed soon after death. Cultures from the larynx, bronchial tubes and lung exudate showed large numbers of moist, spreading, slightly hemolyzing streptococci, a few typical hemolytic streptococcus colonies, and a moderate number of staphylococci, while blood-agar-plate cultures from dextrose broth inoculated with the blood from the heart showed pure growth of moist, spreading, nonhemolyzing green-producing colonies of streptococci. The morphology and character of the colony of the slightly hemolyzing streptococci and the green-producing streptococci from the blood were identical. In another case of influenzal bronchopneumonia with huge right and slight left empyema and beginning pericarditis, the cultures showed large numbers of hemolyzing streptococci and staphylococci from the pneumonia, a few hemolyzing streptococci and staphylococci from the left pleura, staphylococci from the pericardium, and larger numbers of green-producing streptococci and a few staphylococci in the pus from the right pleural cavity.

Staphylococci were rarely found in large numbers in the sputum early in the disease, but there was in general a tendency to an increase in the numbers of these organisms during the later stages, especially of influenzal pneumonia. In many instances the green-producing streptococcal flora noted at the outset when the sputum was mucoid in character was later partially or wholly displaced by staphylococcal flora, as the sputum became more purulent in character. This occurred especially during the later stages of the epidemic waves and in groups of patients who became ill at about the same time. Usually no particular change was noted in the patient's condition as staphylococci became more numerous in the sputum. Staphylococci were often found in predominating numbers or in pure culture in the pus from abscesses in bronchopneumonic areas in cases in which hemolytic or green-producing streptococci were the predominating organism in the pneumonic

exudate remote from these abscesses. In such instances it was impossible to evaluate the exact rôle played by these organisms. In some cases, however, there could be little doubt that they were the cause of death just as was the case in the series of staphylococcal pneumonia that developed during the influenza epidemic at Camp Jackson, as reported by Chickering and Park.[6] In these there was a rapid change for the worse in the patient's condition as the staphylococci appeared in large numbers in the sputum and as the sputum became bloody, although purulent in character. The leukocyte count remained at about the same level or became lower as death occurred from acute hemorrhagic edema, or acute bronchopneumonia in from one to three days. The lung exudate after death showed enormous numbers of micrococci in groups in smears and staphylococci in enormous number usually in pure or in almost pure form in cultures. The freshly isolated organisms from some of these cases were found to be extremely virulent for animals. Intratracheal injection into guinea-pigs produced violent symptoms associated with leukopenia, and frequently death occurred from acute hemorrhagic bronchopneumonia, hemorrhagic edema, and voluminous lungs. The picture in these animals was quite different from that following injection of freshly isolated strains of staphylococcus from furunculosis, and from the abscesses in the lungs of some patients who died later. In the latter, symptoms were slight or absent, leukocytosis developed, recovery was the rule, and while areas of bronchopneumonia were found acute hemorrhagic edema never developed.

Influenza bacilli were isolated from the sputum in large numbers only during the early part of the first wave and almost not at all after that. These organisms were present in only a small number of cases after death, and when found were always in small numbers. That the absence of this organism in the cultures was due to their absence in the material cultured and not to their inability to grow on the medium used is certain. The medium which we used throughout the different waves was found quite efficient for cultivating this organism from swabs of the nasopharynx of influenza patients and from normal persons by Dr. Williams and Dr. Hatfield, working in our laboratory during the fourth wave. It is true, however, that cultures made at the same time on the special mediums which they used for isolating influenza bacilli showed this organism in a somewhat higher percentage of cases. We were especially interested in whether or not influenza bacilli were present in the exudate of the lower respiratory tract, the point of chief

attack, but cultures of sputum at this time again showed influenza bacilli in only a few cases, and in these in small numbers. Smears of the sputum, lung exudate, and tracheal mucus failed to show influenza bacilli when they were absent in cultures.

The results of the injection into animals (mice and guinea-pigs) of the sputum and lung exudate directly or of the primary mass culture in dextrose-blood broth served as an additional check on the cultures, for it was thought that growth of influenza bacilli, if of great significance in the production of symptoms in the disease in our cases, might occur in animals known to be susceptible to these organisms. Moreover, it was thought that a fairly accurate knowledge of the degree of invasive power of the different bacteria might be obtained in this way. A study was, therefore, made of the relative numbers of the different bacteria in the sputum and primary cultures in dextrose broth of sputum which was injected into animals and their relative numbers from the peritoneal and lung exudates of the animals that died. Sixty-eight animals succumbed to the intraperitoneal or intratracheal injection of sputum or of primary cultures from sputum and from lung exudate. Green-producing streptococci were the predominant organisms in the material injected in 68 per cent. of the animals, while after death this organism predominated in the blood, peritoneal or lung exudate in 78 per cent. of the animals. In most of the others hemolytic streptococci, and in a few, staphylococci, were the predominating organisms. Influenza bacilli were not isolated in a single instance, notwithstanding the fact that some of the specimens of sputum injected contained this organism in large numbers. The blood-agar plates made from the peritoneal exudate usually showed a mixture of streptococci and staphylococci in about the same proportions as in the material injected, while the blood nearly always contained pure cultures of streptococci, usually of the green-producing variety. In some animals, however, striking deviations from this rule occurred. Staphylococcus colonies in varying numbers often developed from the peritoneal exudate when the blood-agar plate from the material injected showed only streptococcus colonies. In some instances green-producing streptococci were isolated from the blood of these animals in pure culture, or together with hemolyzing streptococci when pure cultures of hemolyzing streptococci were injected, and vice versa. These findings were noted also when the cultures injected were derived from single widely separated colonies and at the same time in series of animals including several species.

The respiratory infections were of quite different types during several weeks prior to the occurrence of the first epidemic wave, at the height of the waves, as the waves subsided, and for several weeks following. Prior to the first severe outbreak mild attacks of pharyngitis and bronchitis with little fever and with slight or no constitutional symptoms occurred. Cultures from these cases showed a green-producing streptococcal flora in the sputum and throat. At the height of the epidemics, especially the first wave, marked prostration, cyanosis and leukopenia, high fever, and marked tendency to lung involvement with slight injection of pharynx and tonsils dominated the picture. Deaths from acute hemorrhagic edema were relatively common. As the waves subsided the symptoms became less marked, leukopenia was less persistent, deaths from respiratory involvement occurred later, and the lung showed relatively more true consolidation, but symptoms referable to infection of the nose and throat were more pronounced. In such cases it was often difficult to make the diagnosis, and cultures in some showed hemolyzing streptococci from the beginning of the attack. Still later well marked pharyngitis, often associated with follicular tonsillitis, absence of leukopenia or even leukocytosis, became prevalent. Involvement of the lung was now rare and deaths from pneumonia no longer occurred. Cultures from the throat and tonsils of patients in the latter condition showed hemolytic streptococci to be the chief organism.

The technic of making the cultures was uniform throughout the four waves, and the results were recorded according to the scale of 1 to 4, thus affording opportunity to study the changes in the character of the colonies of the different species and changes in their relative numbers as the epidemic waves appeared and disappeared. Each of the four epidemic waves studied ran its course in about six weeks, and the crest was reached in about two weeks; accordingly the results of sputum cultures from cases of influenza and influenzal pneumonia, and the fatal cases, were arranged into three groups of two weeks each, and the average of the three main types of bacteria (green-producing streptococci, hemolyzing streptococci, and staphylococci) determined. The first period comprised the first two weeks, the second the third and fourth weeks, and the third the fifth and sixth weeks of the four waves. The figures representing the average incidence of green-producing streptococci for the three periods of the four waves were 2.6, 2.3 and 2.7; for hemolyzing streptococcus 1.1, 1.4, and 1.4; and for staphylococci 1.8, 2.1, and 2.4, respectively. According to

these figures it is evident that the green-producing streptococcus in the sputum averaged the highest throughout the epidemic waves, and that the hemolyzing streptococcus and staphylococcus while comparatively few early in the epidemic became relatively more numerous as the waves subsided. In the case of cultures after death the figures representing the average for green-producing streptococci for the three periods were 1.5, 2.6 and 1.0, for hemolyzing streptococcus 1.1, 1.7 and 2.5, and for staphylococcus 1.1, 1.1 and 2.1, respectively. According to these figures, the green-producing streptococcus was the chief cause of death during the first four weeks of the waves, and the hemolyzing streptococcus and staphylococcus during the fifth and sixth weeks, or as the waves were subsiding.

MORPHOLOGY, CULTURAL CHARACTERISTICS AND FERMENTATIVE POWERS OF THE STREPTOCOCCI FROM INFLUENZA

Green-Producing Streptococci. — The somewhat peculiar green-producing streptococcus isolated during the first wave has been described (Study 1). The further results, throughout the subsequent waves, have in the main corroborated the earlier findings, although greater differences in cultural characteristics have been noted than were at first apparent. From a study of a large number of cases we have found green-producing streptococci, including pneumococci, to be the most common organism present in influenzal infection. The strains when first isolated usually produced rather moist, spreading, non-adherent greenish colonies on blood-agar plates and a diffuse cloud in glucose broth. Smears of young cultures from these mediums showed gram-positive oval shaped diplococci of quite uniform size, singly, in pairs, and usually in fairly long chains. These were of about the size of pneumococci and were often indistinguishable from them, although chain formation was usually more marked and the capsule less distinct. Smears from older cultures, especially in the deeper layers of tall columns of glucose-brain broth, often showed diplococci of extreme variations in size and shape. During the first wave the colonies were quite moist, usually resembling type III pneumococci, although less mucoid in character; in the subsequent outbreaks, especially during the later stages, they were usually not so moist and were often indistinguishable from pneumococcus colonies. The more moist spreading type of colonies, some resembling Pneumococcus mucosus, were isolated, however, during these waves in some instances. This was true particularly early

in each wave, in severe cases occurring in groups of persons who contracted influenza soon after arriving in Rochester, and who came from the same locality, as well as in individual families residing in Rochester and in the surrounding country. The chief distinguishing characteristics of these strains as of those in the first wave, however, were their marked and peculiar invasive power on intratracheal injection. As the

TABLE 2

THE VARIABILITY IN FERMENTATIVE POWER OF GREEN-PRODUCING STREPTOCOCCI FROM INFLUENZA

Date of Test	Strain	Dextrose	Lactose	Maltose	Saccharose	Raffinose	Mannite	Salicin	Inulin	Control
9/25/18	2539.6	+4	+4	+4	+4	+4	0	+4	0	0
3/ 6/19	2539.8	+3	+2	+2	+4	+4	+	+2	0	0
3/ 4/19	2341.17	+3	+3	+3	+4	+4	0	+3	+	0
12/30/19	2341.20	+2	0	+	+	0	0	0	0	0
3/ 3/19	2347.12	+3	+3	+2	+4	+4	+	+	+	0
11/14 19	2347.23	+3	+3	+4	+3	0	0	0	0	0
3/ 3/19	2349.12	+3	+3	+3	+4	+4	0	+2	+2	0
11/14/19	2349.18	+4	+3	+4	+3	0	0	0	0	0
3/ 6/19	2531.8	+3	+2½	+3	+4	+	0	0	+2	0
11/ 4/19	2531.12	+3	+3	+3	0	0	0	0	0	0
3/ 6/19	2532.0	+3	+3	+3	+4	+4	0	+3	+2	0
11/ 4/19	2532.8	+3	0	+2	+2	+	+3	+3	0	0
3/15/19	2620.3	+3	0	+3	+4	0	0	+4	0	0
3/15/19	2620.3	+2	+	+2	+4	0	0	+3	0	0
3/10/19	2724.8	+3	+2	+2	+4	+4	0	0	0	0
11/18/19	2724.11	+	+3	+3	+3	0	0	0	0	0
3/12/19	2789.4	+3	+3	+2	+4	0	0	0	0	0
12/17/19	2789.8	+3	+	+2	+2	0	0	0	0	0
3/12/19	2748.8	+3	+3	+3	+3	+4	0	+3	0	0
5/ 8/19	2748.10	0	+	+2	+3	+4	0	+	+3	0
11/22/19	2748.11	+3	+	+2	+	0	0	0	0	0
3/12/19	2762.6	+3	+3	+	+4	+4	0	+4	+3	0
11/22/19	2762.8	+	+3	+2	+	+2	+2	0	0	0
3/12/19	2763.6	+3	+3	+3	+3	+4	+2	+4	0	0
10/22/19	2763.14	+4	+	+3	+	0	0	0	0	0
11/22/19	2763.9	+3	+3	+2	+	+3	0	+	0	0
3/12/19	2770².7	+3	+4	+3	+3	+4	0	+3	0	0
3/12/19	2770².7	+3	+3	+3	+3	+4	0	+2	0	0
5/ 8/19	2770².9	+2	+2	+2	+4	+4	+	+4	+2	0
3/19/19	2818.6	+3	+3	+3	+4	+4	+4	+3	+3	0
10/22/19	2818.9	+3	+2	+3	+3	+1	+2	+3	0	0
3/26/19	2824.6	+3	+2	+2	+3	+4	0	0	0	0
11/ 6/19	2824.14	+3	+2	+2	+4	0	0	+3	0	0
3/26/19	2825.6	+3	+3	+3	+4	0	0	0	0	0
4/18/19	2825.11	+3	0	0	+2	0	0	+4	+	0
11/10/19	2825.3	+4	+	+3	+4	0	0	0	0	0
4/16/19	3365.2	+3	+3	+3	+4	+4	0	+3	0	0
4/16/19	3365.2	+4	+3	+3	+4	+4	0	+3	+3	0
11/14/19	3365.7	+3	+3	+3	+3	+	0	+	0	0

epidemics subsided and the infections became milder the colonies of green-producing streptococci from sputum became smaller, and less moist, capsule formation was slight or absent, the chains were longer, and growth in glucose broth was more granular or occurred only at the bottom of the tubes. The virulency of these, determined by intraperitoneal injections into mice and intratracheal injections into guinea-pigs was of a lower order. This difference in character of growth was

TABLE 3

THE VARIABILITY IN FERMENTATIVE POWER OF HEMOLYTIC STREPTOCOCCI FROM INFLUENZA

Date of Test	Strain	Dextrose	Lactose	Maltose	Saccharose	Raffinose	Mannite	Salicin	Inulin	Control
3/15/19	2541.3	+3	0	+2	+4	0	0	+3	0	0
12/12/19	2541.6	+3	+2	+3	+3	0	0	+3	0	0
3/15/19	2559.5	+3	0	+	+4	0	0	+4	0	0
12/12/19	2559.8	+4	+2	+3	+3	0	0	+3	0	0
3/ 6/19	2557.14	+3	+3	+2	+4	0	0	+3	0	0
3/ 6/19	2557.14	+3	+3	+3	+4	0	0	+3	0	0
12/16/19	2557.16	+3	+3	+3	+4	0	0	0	0	0
5/ 8/19	2774.10	+2	0	0	+3	0	0	+2	+3	0
12/19/19	2774.13	+3	+2	+3	+4	0	0	0	0	0
3/19/19	2821.6	+4	+2	+3	+4	0	0	+4	0	0
10/23/19	2821.12	+4	+	+3	+	0	0	+2	0	0
3/21/19	2815.6	+3	0	+2	+4	0	0	+	0	0
12/17/19	2815.28	+3	+2	+2	+3	0	0	0	0	0
3/26/19	2826.6	+2	0	+3	+4	0	+4	0	0	0
10/26/19	2826.5	+4	+3	+	+3	+2	+2	0	0	0
3/26/19	2851.3	+3	+2	+4	+3	0	0	+3	0	0
3/26/19	2851.4	+3	+3	+3	+3	0	+4	0	0	0
12/19/19	2851.6	+3	0	+3	+3	0	0	+2	0	0
4/ 8/19	2902.3	+3	+3	+2	+4	0	0	0	0	0
11/24/19	2902.4	+3	+3	+3	+2	0	0	+4	0	0
4/16/19	3358.2	+3	+3	+3	+3	0	+4	+4	0	0
12/22/19	3358.6	+4	+	+2	+	0	+2	+3	0	0
4/29/19	3387².3	+2	+4	+2	+3	+3	0	+3	0	+
11/14/19	3387².5	+4	+3	+4	+3	0	0	+4	0	0
4/29/19	3395.5	+2	+4	+3	+3	+4	0	+3	0	0
12/19/19	3395.9	+2	+	+	+	0	0	0	0	0
4/29/19	3398.4	+3	+3	+2	+3	0	+2	+3	0	0
5/24/19	3398².5	+4	+2	+2	+4	+4	0	+3	0	0
1/ 3/20	3398.7	+2	0	0	+	0	+2	0	0	0

noted in strains isolated directly from the sputum as well as from the animals that succumbed to injections of sputum. Distinct as these differences were in cultural features, immunologic studies (Study III) showed most of them to be identical, especially those isolated early in the attack. In making cultures from the sputum and lung exudate on blood-agar plates small indifferent colonies of streptococci resem-

bling influenza bacilli were frequently noted. This resemblance was often so marked that examination of smears stained with a Gram stain were necessary to differentiate them. These often acquired the power to produce green on blood agar after one or more cultivations in tall tubes of glucose broth. Moreover, colonies which appeared to be transition forms between green-producing and hemolytic streptococci were frequently noted, especially in the sputum of patients as the epidemics were subsiding. After cultivation on artificial mediums the strains often showed marked changes. The colonies became dry, and smaller; and often diffuse growth in glucose broth no longer occurred, but instead a growth with granular sediment resembling Streptococcus viridans. In some instances indifferent colonies developed and in many instances they acquired hemolytic power. This was of all grades from a narrow zone peripheral to an inner green zone to well marked hemolytic zones beginning immediately around the colony.

Hemolytic Streptococci.—The infections of the lung by hemolytic streptococci, as they occurred during the pandemic of influenza usually without empyema, without tonsillitis and without leukocytosis, but with leukopenia, indicated peculiar infecting powers not possessed by hemolytic streptococci found so commonly in normal throats and tonsils in acute follicular tonsillitis, and in the pneumonia empyema epidemic of 1917-1918. This has been found actually to be the case. Strains from the sputum and lung in cases of acute hemorrhagic edema reproduced this condition associated with leukopenia in guinea-pigs on intratracheal injections; whereas strains of hemolytic streptococci from simple pharyngitis caused leukocytosis (reported elsewhere), but never acute hemorrhagic edema of the lung. Culturally, there was often a distinct difference between these strains and those isolated from cases of empyema and the throats of persons suffering from pharyngitis and tonsillitis. The colonies were more moist, less opaque, often spreading in character, quite as the colonies of green-producing streptococci, and the hemolytic zone was not so wide, not so clear, and the margin less sharply defined. Freshly isolated strains from acute cases produced diffuse growth in glucose broth, while hemolytic streptococci from the pus in cases of longer duration like those from other sources, usually grew granular with flocculent sediment. Morphologically, these strains were at times indistinguishable from the green-producing streptococcus, the smears showing elongated diplococci singly and in chains of various lengths. After cultivation on artificial

mediums for a time these peculiar properties tended to disappear, in some instances abruptly, and were no longer distinguishable from the hemolytic streptococcus obtained from other sources. In some instances the hemolytic streptococci acquired the power to produce green colonies.

The tendency of the green-producing streptococci to acquire hemolytic powers, and to a lesser degree the tendency of the hemolytic streptococci to acquire the power to produce green colonies was so marked that it was found necessary in agglutination experiments to plate the cultures actually agglutinated in order properly to interpret the results obtained. The tendency of hemolytic streptococci to lose their hemolyzing powers was especially marked in freshly isolated cultures when grown in tall tubes of glucose-brain broth. This frequently occurred in cultures derived from single colonies from plates showing large numbers of the organism in question in pure form. The cultures which were put aside for study were made on blood-agar slants from single colonies, or from a group of colonies well separated from other bacteria. The tendency to mutations in these cultures may best be illustrated by giving the results of subcultures when this point was especially noted. Thus of a total of 623 cultures, green-producing streptococci bred true to type in 348 instances, and hemolytic streptococci in 168, a total of 516. In the remaining 115 cultures, green-producing streptococci yielded hemolytic streptococci in 45 instances, indifferent streptococci in 27, and staphylococcus colonies in 22, while hemolytic streptococci yielded green-producing streptococci in 9 instances, indifferent streptococci in 7, and staphylococci in 5. In most instances the changes in type occurred abruptly, under various conditions, but especially when old cultures on blood-agar and dextrose-brain broth were transferred. The instability of the streptococcus strains from influenza often made it difficult to obtain the proper proportions of the different strains in the vaccine which was used for prophylactic inoculations. The routine procedure consisted of transferring single colonies or a group of colonies of the different bacteria to bottles containing 150 c c of 0.2 per cent. glucose broth, incubating these over night, making smears, plating a loop full, and inoculating about 30 c c with a bulbed pipet into large bottles containing about 3,500 c c of glucose broth. Smears and blood-agar plates were again made of the latter. It frequently happened that while the plating from the small bottles of broth showed pure growths of the type inoculated, the plating from the large bottle often showed a partial or totally changed streptococcus flora, and not infrequently showed staphylo-

coccus colonies as well, in spite of great precautions taken to avoid accidental contamination. Owing to these findings, further studies were made on this point. The findings in Case 3101 and the behavior of the strain isolated will suffice to illustrate results obtained along this line:

Case 3101, a man, aged 32, became sick Feb. 28, 1919, with severe headache, aching all over the body, marked prostration and chilly sensations, but no distinct chill. The next day he was admitted to the hospital with a temperature of 104, pulse 90, and respiration 20. The throat was slightly infected, and moderate dullness and decreased breath sounds over the base of both lungs were found. The temperature ranged between 100 and 104 until March 5. March 6 the temperature was higher and scattered areas of dullness with râles were elicited. March 7 the patient became cyanotic, extremely short of breath, and expectorated large amounts of bloody, frothy material. He grew rapidly worse in spite of venesection (400 c c) and transfusion of 250 c c of convalescent human blood, and died March 8. The leukocyte count on March 3 was 10,600; March 4, 9,000; March 5, 9,500; March 7, 9,100. March 2 and 6 the sputum was mucopurulent and showed large numbers of green-producing streptococci, slightly hemolyzing streptococci, and staphylococci. March 7, the day the patient became worse, the sputum showed no change in flora, but a blood culture yielded a pure growth of hemolytic streptococci. March 8 the sputum contained enormous numbers of hemolytic streptococci and staphylococci, but no longer green-producing streptococci nor slightly hemolyzing streptococci. One guinea-pig was injected with the twenty-four hour primary culture from the blood in glucose-brain broth. The animal was ill for a time and lost 30 gm. in weight, and then recovered. A second guinea-pig was injected March 9 with 2 c c of the glucose-brain broth culture made from a single colony of hemolytic streptococcus on a blood-agar plate inoculated with the culture injected into the first guinea-pig. The plate showed a pure culture of hemolytic streptococcus. The guinea-pig lost in weight, and respirations were increased. It was chloroformed two days after injection and two large areas of consolidation in the right diaphragmatic lobe were found. From these areas pure cultures of green-producing streptococci, but no hemolytic streptococci were obtained. The tube of glucose-brain broth inoculated with the blood, which showed on plating a hemolytic streptococcus in pure form, was placed in the ice chest until September 8, when a glucose-brain-broth culture and a plating on blood-agar were made. The blood-agar plate yielded one colony of hemolytic streptococcus. From this colony a blood-agar plate and a tall tube of glucose-brain broth were inoculated (September 13). The former showed pure cultures of hemolyzing streptococci. The latter developed abundant growth, but the tube was not opened until November 28, at which time two blood-agar plates were made; both showed many colonies of hemolyzing streptococci as well as green-producing streptococci. From this plate single hemolyzing and green colonies well separated from other colonies were inoculated December 1 into one tube of glucose-brain broth each. Both tubes developed abundant diffuse growth, and platings on blood-agar December 2 showed pure cultures of green-producing streptococci. In both instances intratracheal injection into 3 guinea-pigs of the growth in the tube inoculated with hemolytic streptococcus colonies resulted in the production of acute bronchopneumonia with death of two on the third day while the third animal recovered after three days of illness. Green-producing streptococci were isolated in large

numbers from the two that died. Hemolytic streptococci in the tube of glucose-brain broth when fresh were injected intratracheally into one guinea-pig. It developed bronchopneumonia and from the lung lesions pure cultures of green-producing streptococci were isolated. The tube of glucose-brain broth inoculated (September 9) showed diffuse growth, and a blood-agar plate September 10 contained pure culture of typical hemolytic streptococci. A single colony was used to inoculate a blood-agar plate and a tube of glucose-brain broth. The former yielded a pure culture of hemolytic streptococci; a plating of the latter September 13 also showed a pure culture of the hemolytic streptococcus, but a plating made November 28, after incubation at 35 C. since September 9 showed countless numbers of indifferent colonies of streptococci and moderate numbers of slightly hemolytic streptococci. The tube of glucose-brain broth inoculated with a single colony September 10 developed diffuse turbidity and September 12 yielded countless hemolytic streptococcus colonies on blood-agar plates. A blood-agar plate made of this same tube November 28 showed countless staphylococcus colonies with no hemolytic or green-producing streptococcus colonies. December 2 intratracheal injection into two guinea-pigs of this culture containing staphylococci caused leukopenia, increased respiration for a time, and death on the eleventh day. There was no lung involvement and the organism was lost. The culture in glucose-brain broth made from a single colony of hemolytic streptococci September 13 was injected intratracheally into one guinea-pig and intraperitoneally into a mouse. The guinea-pig had marked increased respiration for a number of days, and died nineteen days after the injection from interstitial bronchopneumonia. The mouse lived thirteen days, and then died. Cultures of the blood of both yielded hemolytic streptococci.

The infecting power and immunologic conditions of the organisms as changes occurred have been studied extensively. The details of these experiments will be reported elsewhere, but it may be stated that high and peculiar invasive powers of the changed forms have been noted repeatedly, and that as cultural properties changed immunologic reactions usually became different also.

FERMENTATIVE POWERS OF THE STREPTOCOCCI

The fermentative powers of the streptococci over the usual test sugars have been determined in a large number of strains. The method which we have found most efficient and convenient for this study is a modification of the Hiss serum water medium. This modification consists of the Hiss serum water medium with the addition of 0.5 per cent. agar, just sufficient to jell, 1 per cent. of the different test sugars, and Adraid's indicator instead of litmus. The medium is placed in small tubes, 3 inches by $\frac{3}{8}$ of an inch, about 2 c c into each tube, and steamed in the usual manner on three successive days. Inoculations are made by stabbing the medium with a loop containing organisms from fresh cultures on blood-agar. The tubes are incubated for seventy-two hours, and then read. Negative reactions can be deter-

mined easily since if growth has taken place a streak along the line of inoculation can readily be made out. A negative result is not recorded unless a distinct growth has taken place. The degree of acidity is indicated with one or more + signs according to the depth of red color produced.

Altogether, we have tested the fermentative power of 254 strains of the green-producing streptococci soon after isolation. Of these, 94 per cent. fermented dextrose; 90 per cent. lactose; 93 per cent. maltose; 78 per cent. saccharose; 49 per cent. raffinose; 35 per cent. mannite, 67 per cent. salicin, and 38 per cent. inulin. After cultivation for from six to nine months on blood-agar, 139 strains were again tested. The results in dextrose, lactose, maltose and saccharose were practically the same as with the freshly isolated strains, but the number fermenting raffinose, mannite, salicin and inulin was decidedly less in each, the percentage being 22, 16, 45 and 17, respectively. Tests of the fermentative power of 119 strains of hemolyzing streptococci soon after isolation resulted as follows: 91 per cent. fermented dextrose, 71 per cent. lactose, 87 per cent. maltose, 82 per cent. saccharose, 19 per cent. raffinose, 16 per cent. mannite, 79 per cent. salicin, and 8 per cent. inulin. After prolonged cultivation a general lowering of fermentative powers was noted in 70 strains tested. This was especially marked in the case of lactose and salicin. The instability of these strains as noted on blood-agar and other mediums was noted also with respect to their fermentative powers. The results obtained from a study of a large series of strains by testing each strain on different dates are well illustrated by the summaries in tables 2 and 3. It becomes apparent at once from a study of these tables that a classification of the streptococci, especially the green-producing streptococci, on the basis of their fermentative reactions would have little real meaning, since they frequently acquire or lose, quite without regard to rule, the power to ferment important carbohydrates.

GENERAL DISCUSSION AND SUMMARY

From a bacteriologic study of a large series of cases of influenza and influenzal pneumonia throughout four epidemic waves, green-producing streptococci (including pneumococci) were found to occur more constantly and in larger numbers than any other organisms commonly associated with this disease. This flora predominated alike in the cases of influenza without lung involvement, in those of lung

involvement in the initial febrile attack as well as in those in which influenzal pneumonia developed after a quiescent interval following influenza. This was especially true early in the attacks throughout the epidemic waves, and the flora usually persisted and was the chief cause of death during the early part and during the height of the epidemic waves. Moreover, the agglutination experiments with a monovalent immune horse serum have shown that most of the strains isolated early in the disease are immunologically alike, whereas later they become more heterogeneous, just as do the specific strains after cultivation on artificial mediums.

During the latter part of the outbreaks hemolytic streptococci became relatively more numerous, especially late in the disease, and death was often the result of invasion by these organisms. A similar increase in the number of staphylococci occurred, and in some instances these appeared to be the immediate cause of death. The change in the type of the disease and the character of the lesion in the lung, which were noted during each of the epidemic waves, appeared to be due more to a change in the virulence of the organisms than to a change in the type of flora. Thus well marked instances of acute hemorrhagic edema occurred, but almost wholly at the height of the epidemic waves in which each of these organisms was found in pure culture or in mixture in various proportions.

The influenza bacillus was found in the sputum in the early part of the first wave only in a few cases and always in association with streptococci, while throughout the remaining three waves it was isolated only occasionally. The criticism which has been raised by those who believe the influenza bacillus to be the cause of influenza, that the methods used by those who fail to isolate this organism are inadequate, does not apply to this study, because the medium used throughout this study was proved effective for the growth of the influenza bacillus. Special mediums were employed during the latter part of the study, smears failed to show the organism, and influenza bacilli were not found in the animals injected directly with sputum and lung exudate. Hence it is certain that in the majority of cases studied this organism played little or no rôle in the production of symptoms. Its presence in large numbers in some cases in the early part of the first wave and its almost complete absence in sputum and lung exudates subsequently indicate that the difference in the frequency of isolation of this organism by various workers is in general, as emphasized by

MacCallum and others, a measure of its prevalence in the particular epidemics studied, and that many epidemics of typical influenza occur that are not due to this organism. Moreover, when found it is usually associated with organisms of the pneumococcus-streptococcus group (Park, Williams, Dick and Murray).

The finding of a preponderance of the pneumococcus-streptococcus group of organisms reported herewith is in accord with the results obtained by the pneumonia unit at Camp Lewis, by Blanton and Irons, Friedlander, McCord, Sladen and Wheeler, Stone and Swift, Jordan, Hirsch and McKinney, Dunn, and many others.

It has been demonstrated repeatedly in this study that the peculiar infecting power of streptococci from influenza does not depend on their power to ferment certain carbohydrates, and that the fermentation reactions are variable. Hence the difference in the relative number of instances in which green-producing streptococci, hemolytic streptococci, or Group 4, or even type pneumococci were isolated by the different workers during the pandemic does not necessarily mean that the different strains did not have the infecting power peculiar to influenza, just as has been found to be the case in our hands. A striking example in support of this idea occurred at Camp Grant in which type II pneumococci of extreme virulency were found to be the cause not of lobar pneumonia, but of acute bronchopneumonia typical of influenza during an extremely fatal epidemic as described by Hirsch and McKinney.

The changes observed in morphology, cultural characteristics, fermentative and immunologic reactions in the green-producing streptococci indicate that the organism described by the English observers and designated by them as diplostreptococcus, the green-producing streptococcus found by Mathers as described by Tunnicliff, the diplococcus epidemicus described by Bernhardt and by Segale, the diplococcus mucosus described by Stephan, and the pleomorphic streptococcus described by Wiesner in influenza, are identical with the green-producing streptococcus isolated in this study, or modifications thereof. Moreover, the marked changes or true mutations that have occurred in the culture tube under controlled conditions indicate that the change in the bacterial flora at different stages of the disease in the individual and in the epidemic waves may not always be the result of superimposed infections from the upper respiratory tract as is now generally believed.

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