AN EPIDEMIC OF PNEUMOCOCCUS BRONCHO-PNEUMONIA *

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INTRODUCTION

An epidemic disease characterized by a sore or dry throat, cough, fever, general prostration, and in a certain number of patients by a rapidly developing pneumonia broke out at Camp Grant, Ill., Sept. 21, 1918. The hospital admissions increased rapidly, attained their maximum between Sept. 26 and Oct. 2, and then gradually diminished until by Oct. 18, they had reached about the usual daily hospital admission rate. During the period between Sept. 21 and Oct. 18, 9,037 patients were admitted to the hospital. While all of the admissions were not the direct result of the epidemic, the proportion of those not so entering was extremely small. During the several days immediately preceding the onset of the epidemic a few patients, not more than 15 or 20, were admitted with the diagnosis of influenza, and after Oct. 18 others were admitted with a similar diagnosis, but not in as large numbers nor as seriously ill, and during the epidemic proper some of the mildly ill patients were treated and quartered in the infirmaries. The number of hospital admissions mentioned, therefore, does not account accurately for all those sick with the epidemic disease.

The disease began suddenly, commonly within a period of 10-12 hours from a state of comparative well being. Some patients said that they had coughed or had had a cold for several days preceding the acute onset of illness. Prostration was a striking manifestation, many coughed, all had fever, and those with moderately severe nasopharyngeal or bronchial manifestations had a peculiar dusky, almost cyanotic, flushed face, particularly the forehead, cheeks, and lips.

Pneumonia rapidly became the serious consequence in the epidemic, and its high mortality one of the most baffling factors so far as the medical management of patients was concerned. Many patients were in the hospital but a short time before death, and during this interval had extreme respiratory difficulty, with marked cyanosis of the face and body generally. Some became moderately or intensely jaundiced early in the disease, a circumstance that

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suggested strongly a blood invasion by some pathogenic organism. The total number of pneumonia patients registered in the hospital from Sept. 20 to Oct. 17 is 2,371.

The first death occurred on the 3rd day after the explosive onset of the epidemic in a patient with a diagnosis of pneumonia. From Sept. 21 to Oct. 18 there were 1,019 deaths, and from the latter date to Oct. 31 there were 51. Almost every death resulted from pneumonia, although during the latter part of the epidemic, complications and sequelae of an active or healing pneumonia contributed no small part in continuing the mortality. In addition to these, infections of the leptomeninges, sinuses, and other bony spaces of the head became of decided importance.

The arrival of a large number of patients in the hospital with symptoms referable to infection of the respiratory passages suggested at once that the possible or even highly probable portal of entry of the disease-producing organism was the nasopharynx, and that a bacteriologic examination of the nasopharynx would demonstrate the variety of bacteria as well as the predominating organism, if there were any such. This contention was further supported by the fact that the pharyngeal tissues were edematous, reddened, and otherwise obviously involved in the infection.

From about the first 300 patients admitted, throat cultures were made on Loeffler's medium, and having determined after 24 hours' incubation that the predominating organisms growing on this medium were gram-positive, usually diplococci as such or in short chains, about 80 throat cultures were made as surface streaks on dextrose blood-agar plates. This medium, it was thought, would aid materially in differentiating the gram-positive organisms, as well as furnish a favorable substrate for the growth of such gram-negative bacilli as B. influenzae. In order to make this contention more secure, if possible, another series of surface inoculations was made on plain blood-agar plates from about 90 more patients. All of these cultures were made on the second and third days of the epidemic — practically at its onset.

Appreciating further that a pneumonic process in all probability would follow in a certain number of patients, and that careful bacteriologic examinations would be vitally important in the postmortem studies, a systematic plan of investigation was laid out and followed rigidly. This plan is detailed as followed:

1. To culture in dextrose broth, blood removed from the heart with a sterile pipet.

2. To inoculate in surface streaks on plain blood-agar plates the exudates from consolidated lung tissue and from bronchi.

3. To make direct smears on glass slides of such exudates for comparison with (2), stained according to the Gram's method (counterstained with dilute aqueous fuchsin).

4. To culture pleural, pericardial, spinal, and other fluids or exudates in dextrose broth or on plain blood-agar plates.

5. To make surface streaks on plain blood-agar plates of exudates found in the sinuses of the head or middle ears.

6. To isolate in pure culture and identify the organisms found in the diseased tissues, and in the various body fluids and exudates in so far as is possible.

TABLE 1

PNEUMOCOCCI FROM THROAT CULTURES

Culture	Inulin Fermentation	Capsule		Bile Solubility	Amount of 24-Hour Blood-Agar Slant Injected into White Mouse	Dose Fatal in Hours	Type	Organism Recovered Pure from Mouse Heart Blood
		On Blood Agar	In Heart Blood of Mouse					
4	—	—	+	+	0.166	24	IV	+
6	—	+	+	+	0.166	34	II	+
8	—	—	+	+	0.166	20	II	+
11	—	—	+	+	0.166	18	IV	+
12	—	—	—	+	0.166	21	IV	+
15	—	—	—	+	0.166	20	IV	+
16	—	—	+	+	0.166	20	II	+
18	+	—	—	+	0.166	18	IV	+

+ positive; — negative.

7. To fix in Zenker's solution tissues for microscopic study, as well as to preserve gross specimens for museum demonstration.

8. Having determined the predominating organism, to test its pathogenicity in susceptible, moderately susceptible, and resistant laboratory animals.

This much of a plan was laid down early, and carefully followed. Later additional methods of investigation were developed, among which are blood cultures of patients in the hospital. In the cultures of all exudates, recently prepared, plain blood-agar plates were used, and usually controlled by direct smears on glass slides stained by Gram's method.

The isolation and identification of bacteria were carried out according to recognized standard methods, the details of which appear later.

THROAT CULTURES

The frequency of nasopharyngeal inflammation in the early admissions to the hospital prompted the making of cultures on Loeffler's medium from the first 297 patients received. These cultures were taken in the usual way with

sterile cotton applicators, the secretions collected on the cotton inoculated at once on the surface of the medium. After about 24 hours' incubation, smears of the surface growth were made on glass slides, fixed, and stained according to Gram's method, counterstaining with a dilute aqueous solution of fuchsin. The results of the microscopic examination of these cultures are given in the following table according to the predominating or mixed flora noted:

Gram-positive diplococcus	189
Gram-positive diplococcus and staphylococcus	6
Gram-positive diplococcus and gram-negative bacillus	1
Gram-positive diplococcus and gram-negative coccus	4
Gram-positive diplococcus and gram-positive bacillus	3
Staphylococcus	30
Gram-negative bacillus	1
Gram-negative coccus	6
Gram-negative bacillus and gram-positive bacillus	1
Gram-positive bacillus	8
No growth	48

This early survey demonstrated a remarkably high frequency of gram-positive, frequently lancet-shaped diplococci as such or in short chains in cultures from the posterior pharynx of these patients. It also disclosed a relative infrequency of gram-negative organisms of all kinds, presuming of course that the substrate favored their growth. Not feeling entirely certain of this, and wishing to differentiate still further the variety of gram-positive organisms on culture mediums, a series of 71 throat cultures was taken on dextrose blood-agar plates. This was done on the second and third days of the epidemic. After 24 hours' incubation practically every plate contained fine, moist, transparent or nearly so, slightly umbilicated colonies of about ½ mm. diameter surrounded by or contained in a distinct zone of green. Many of these plates were pure cultures of such colonies containing gram-positive diplococci, commonly lancet-shaped, and sometimes arranged in short chains. Another series of 88 plain blood-agar plates was prepared from other patients in the hospital, and these also contained many fine green colonies, frequently such almost exclusively. All of the blood-agar plates were searched carefully for gram negative bacilli, but these were found only occasionally, and never as a predominating organism in any culture.

With the purpose of determining more closely the presence of gram-negative bacilli in throat cultures, sterile cotton applicators used to collect the secretions from the posterior pharynx were shaken out in broth tubes, and from the broth dilution plain blood-agar plates were poured. Fourteen such plates were made, each one controlled by surface inoculation on plain blood agar plates of the undiluted secretions, and colonies of gram-negative bacilli were found in no large numbers in three, and always in plates where the predominating organism was a gram-positive diplococcus.

Colonies of hemolytic streptococci appeared occasionally in 20 of the 159 blood-agar plates inoculated with secretions from the posterior pharynx, nonhemolytic in not more than 13. During the examination of the throat cultures on blood-agar plates, many strains of gram-positive diplococci were isolated, and table 1 lists a few, giving their morphology, cultural characteristics, type, and approximate virulence.

ANATOMIC CHANGES

Early involvement of the lungs seemed an inevitable consequence with an upper respiratory tract infection accompanied by such severe prostration, and as a matter of fact, patients came to the hospital with a well developed pneumonia. On the third day of the epidemic proper the first death occurred with the diagnosis of pneumonia and with few exceptions this was the clinical diagnosis of other patients dying in the hospital during the epidemic. Those others not so diagnosed died from sequelae or complications of a lung infection, or from complications of a cranial sinus infection or middle ear disease.

TABLE 2

PNEUMOCOCCI FROM HEART BOOD POSTMORTUM

Culture	Inulin Fermentation	Capsule		Bile Solubility	Amount of 24-Hour Blood-Agar Slant Injected into White Mouse	Dose Fatal in Hours	Type	Organism Recovered Pure from Mouse Heart Blood
		On Blood Agar	In Heart Blood of Mouse					
1001	+	—	+	+	0.0231	11	II	+
1002	+	—	+	+	0.0231	11	II	+
1005	+	—	+	+	0.0231	17	IV	+
1011	+	—	+	+	0.0231	23	II	+
1013	+	—	+	+	0.0231	18	IV	+
1016	+	—	+	+	0.0231	13	IV	+
1017	+	—	+	+	0.0231	11	II	+
1019	+	—	+	+	0.0231	13	II	+
1020	+	+	+	+	0.0231	11	II	+
1021	+	—	+	+	0.0231	11	IV	+
1037	—	—	+	+	0.0231	27	II	+
1038	+	+	+	+	0.0231	25	II	+
1040	+	—	+	+	0.0231	25	IV	+
1048	+	—	+	+	0.0231	36	IV	+
1049	+	—	+	+	0.0231	16	II	+
1057	+	—	+	+	0.0231	32	I	+
1061	+	—	+	+	0.0231	32	II	+
1066	+	—	+	+	0.0231	24	I	+
1069	+	—	+	+	0.0231	18	II	+
1072	+	—	+	+	0.0231	24	II	+

+ positive; — negative.

During the epidemic 198 postmortem examinations were made. Those held at the beginning included an examination of all the organs of the chest and abdomen as well as the head, but later when the death rate was highest only the organs of the chest were examined. The changes in those dying late in the epidemic deviated only in degree and progress rather than in kind from those observed earlier, and in general, excepting for the organs of the chest, the changes noted early in the epidemic remained the same.

A decided cyanosis of the face, neck and extremities was observed frequently, and commonly there was a distinct yellow tinge to the skin and conjunctivae. Dryness of the skeletal muscles was usual. The parietal pleura commonly was hyperemic and glistening, or slightly dull with loss of the normal smoothness and covered by a scanty layer of fibrin. Turbid brown fluid from a few c c to 300 c c was contained in each pleural cavity when the

inflammation had penetrated into these serous spaces. Changes in the pericardial sac consisted largely in an increase in the amount of yellow fluid normally contained. A true sero-fibrinous or suppurative pericarditis was not seen until late in the epidemic, and acute changes in the heart valves were not observed in any postmortem examination. The myocardium, and all the parenchymatous organs as well, presented a moderate or severe grade of cloudy swelling with commonly disseminated areas of acute fatty changes. Subserous petechial hemorrhages were common.

The parabronchial lymph glands constantly were enlarged, very soft, sometimes with small areas of necrosis. The lining of the trachea and the main bronchi was intensely hyperemic, and on the mucous membranes there was a thin milky, gray, brown, or sanguineous fluid, sometimes frothy. In the deeper portions of the respiratory tree the bronchioles were lined by an intensely red mucous membrane covered by a relatively thin, gray, exudate. The consolidation of the lungs was extensive. When removed from the chest they appeared voluminous, dark red or cyanotic, with no or very little exudate on the pleural surface, these membranes commonly being quite smooth and glistening. Underneath the pleura there were hemorrhages into the lung tissue from one to several centimeters extent as a rule, in addition to nodular consolidations which sometimes were so extensive as to form large confluent areas of firm tissue. Such extensive consolidation usually occurred in those patients that had survived several days, those in whom the lungs were extremely hemorrhagic dying relatively early in the disease. Several of the lobes were involved. From the surfaces of the lung tissue large quantities of blood and bloody fluid escaped, and there were firm areas of red tissue slightly granular and moist, with edematous and hemorrhagic lung tissue between. As the epidemic progressed gray nodular consolidations often confluent were noted. On the cut surface the tissue was distinctly granular and moist with a brown or brownish red exudate.

The yellow of the adrenal cortices was moderately diminished, or entirely absent and in one case there were several small subcapsular hemorrhages in each organ. As a rule the spleen was increased in size from one and one-half times to twice the normal, and the substance of the organ was soft and dark red. The biliary and mesenteric lymph nodes were constantly increased in size, the former much more so relatively than the latter, the substance moderately firm and red. Changes in the common bile duct, the portal vein, the splenic, and the upper portion of the superior mesenteric veins were not seen.

The liver was enlarged, its capsule tense and the tissue beneath mottled with disseminated areas of acute fatty changes and passive hyperemia. Focal necrosis was not recognized grossly. In the lining of the stomach there were multiple petechial hemorrhages.

In a limited number of cases in which the brain was examined there was found a moderate hyperemia of the pia-arachnoid, with dryness of the cerebral cortex. The spinal fluid of such patients usually without meningeal manifestations, commonly was turbid. In a limited number of examinations collection of a mucopurulent exudate was found in the sinuses of the face and middle ears.

As the epidemic progressed, complications commonly following pneumonia were found post mortem, including serofibrinous pleuritis, empyema of the chest, acute serofibrinous pericarditis, acute suppurative pericarditis and peritonitis. The character of the lung changes varied too in the later stages,

and it was in the last few days of the epidemic that gray consolidations of the lungs were noted, and in a few of these extensive softening of the lung tissue. In some, lung changes grossly corresponded with descriptions of acute suppurative interstitial pneumonia. Mention should be made of cases in which the lung changes were comparatively insignificant, but in which disease of some of the bony spaces of the head occurred and subsequently or coincidently infection of the leptomeninges. In these the greatest interest centers in the bacteriologic examinations which are discussed later.

TABLE 3

PNEUMOCOCCI FROM LUNG CULTURES POSTMORTUM

Culture	Inulin Fermentation	Capsule		Bile Solubility	Amount of 24-Hour Blood-Agar Slant Injected into White Mouse	Dose Fatal in Hours	Type	Organism Recovered Pure from Mouse Heart Blood
		On Blood Agar	In Heart Blood of Mouse					
200	+	—	+	+	0.0231	19	II	+
207	+	—	+	+	0.00925	20	II	+
208	+	—	+	+	0.00925	20	II	+
210	+	—	+	+	0.0055	24	IV	+
21	+	—	+	+	0.0055	36	IV	+
221	+	—	+	+	0.0055	18	IV	+
226	+	—	+	+	0.0055	36	II	+
230	+	—	+	+	0.0055	24	II	+
231	+	—	+	+	0.0055	26	IV	+
251	+	—	+	+	0.0154	20	IV	+
259	+	—	+	+	0.0055	18	II	+
366	+	—	+	+	0.00185	34	II	+
273	+	—	+	+	0.0231	12	IV	+
296	+	—	+	+	0.0154	12	II	+
312	+	—	+	+	0.00925	20	II	+
316	+	—	+	+	0.0055	20	II	+
364	+	—	+	+	0.00185	30	IV	+
365	+	—	+	+	0.00155	30	II	+
266	+	—	+	+	0.0231	12	IV	+
369	+	—	+	+	0.00185	36	II	+
374	+	—	+	+	0.0055	30	II	+
404	+	—	+	+	0.0037	30	II	+
405	+	—	+	+	0.0037	30	II	+
425	+	—	+	+	0.0055	18	IV	+
434	+	—	+	+	0.0055	28	IV	+

+ positive; — negative.

Following the decline of the epidemic, deaths occurred in the hospital fairly often with diseases primarily pulmonary, the lung changes of which differed from those observed during the epidemic. Usually such deaths followed an illness in the hospital of more than one week. In the lungs of those dying within the first or second week there were red or reddish-brown nodular peribronchial consolidations irregularly scattered throughout one or more lobes. The bronchioles contained a white, viscid exudate, the lining membrane being hyperemic and swollen. As this process became moderately advanced, the lungs contained many soft, yellow, peribronchial areas of necrotic tissue from 1-3 mm. in diameter, contrasting sharply with the red, air-containing or compressed lung tissue. Removal of the exudate from these areas exposed shallow crater-like, peribronchial excavations in the tissue, without a definite limiting membrane, being covered by a yellow shaggy exudate. The tissue beneath the exudate was hyperemic or hemorrhagic. Lungs in which this peribronchial destruction of tissue was general were riddled by many abscesses

of relatively small dimension without a definite limiting membrane. Such lobes sometimes were just masses of necrotic tissue held together by the supporting framework.. Other lungs not as extensively diseased contained several or many scattered peribronchial abscesses of relatively small dimension having a thin pyogenic membrane.

Clinically, the patients with extensive destruction of the lung tissue expectorated huge quantities of yellow exudate and liquefied necrotic tissue, a phenomenon regarded by some as the emptying of an empyema through an eroded bronchus. The postmortem examination demonstrated clearly that the yellow expectoration came from the lung itself and not from the pleural cavity.

This acute suppurative interstitial pneumonia, however, was not the pneumonia that characterized the epidemic proper.

Microscopic examination of tissue from various places in diseased lungs disclosed irregularly distributed and developed inflammatory changes. In the alveolar spaces over small or wide areas corresponding with the gross changes, were red corpuscles in large numbers and leukocytes in the ratio usually in the circulating blood. Such areas were distributed irregularly. Other alveolar spaces either in areas of hemorrhage or elsewhere contained endothelial cells in addition to the leukocytes the number of endothelial cells increasing apparently with the progress of the lung lesion. Delicate strands of fibrin were present in such alveolar spaces, and also polymorphonuclear leukocytes which gradually approached and exceeded the endothelial cells in number. In later stages the alveolar spaces contained numerous polynuclear leukocytes, slightly less numerous endothelial cells, fewer red corpuscles, strands and masses of fibrin, cellular detritus, and amorphous particles of coagulated material.

In large confluent areas of consolidation the alveolar spaces regularly contained polynuclear leukocytes, endothelial cells, red corpuscles, and granular protein material as mentioned.

The bronchioles invariably contained many polynuclear leukocytes, endothelial cells, red corpuscles, fibrin, and other products of an acute inflammatory reaction.

CULTURES AT POSTMORTEM EXAMINATION

Of great importance are the results of bacteriologic examinations of diseased tissues, exudates, and fluids removed at the postmortem examinations. Laying aside for the moment all consideration of the predisposing factors of the disease, is it not fair to say that the power of blood stream invasion that an organism possesses determines in a large measure its pathogenicity for the host, and that the fairly constant isolation of the same organism from the blood stream of similarly diseased patients whether during life or soon after death determines with great probability the organism directly responsible for the disease or its fatal issue? Recognizing also in specific diseases that the causative organism frequently manifests a specific election for certain tissues, is it not likely that proper bacteriologic examinations of such diseased tissues in a sufficiently large number will determine the organism or organisms responsible for the local manifestation of the disease?

TABLE 4

PNEUMOCOCCI FROM MISCELLANEOUS CULTURES

Culture	Inulin Fermentation	Capsule in Heart Blood	Bile Solubility	Amount of 24-Hour Blood-Agar Slant Injected into White Mouse	Dose Fatal in Hours	Type	Organism Recovered Pure from Mouse Heart Blood	Source of Culture
2003	+	+	+	0.0154	20	II	+	Pericardial sac
2005	+	+	+	0.0154	12	II	+	Pericardial sac
2006	+	+	+	0.00925	18	1	+	Pericardial sac
2014	+	+	+	0.0154	50	I	+	Pericardial sac
2016	+	+	+	0.0154	48	II	+	Pericardial sac
2052	+	+	+	0.00925	17	I	+	Knee
2082	+	+	+	0.00925	32	IV	+	Knee
2075	+	+	+	0.00925	20	IV	+	Muscle abscess
2077	+	+	+	0.00925	32	IV	+	Muscle abscess
2010	+	+	+	0.0154	40	IV	+	Pleural fluid
2012	+	+	+	0.00925	28	IV	+	Pleural fluid
2013	+	+	+	0.00925	40	IV	+	Pleural fluid
2015	+	+	+	0.0154	72	IV	+	Pleural fluid

+ positive; — negative.

TABLE 5

PNEUMOCOCCI FROM SPINAL FLUID

Culture	Inulin Fermentation	Capsule in Heart Blood	Bile Solubility	Amount of 24-Hour Blood-Agar Slant Injected into White Mouse	Dose Fatal in Hours	Type	Organism Recovered Pure from Mouse Heart Blood
2004	+	+	+	0.00925	18	IV	+
2009	+	+	+	0.0154	30	II	+
2017	+	+	+	0.0154	18	I	+
2011	+	+	+	0.0154	50	I	+
2018	+	+	+	0.0154	42	I	+
2019	+	+	+	0.00925	18	II	+
2055	+	+	+	0.00925	36	II	+
2057	+	+	+	0.00925	18	II	+
2078	+	+	+	0.00925	15	IV	+
2086	+	+	+	0.00925	30	II	+
2039	+	+	+	0.00925	36	II	+
2042	+	+	+	0.00925	16	II	+
2043	+	+	+	0.00925	17	II	+

+ positive.
The last 3 cultures were obtained during life, the others after death.

Accordingly, heart blood was removed with a sterile pipet at each postmortem examination, inoculated into dextrose broth, and after 24 hours' incubation examined for organisms by smears and by streaking out on plain blood-agar plates (0.5-0.7% acid to phenolphthalein). Cultures were made also from the consolidated lung tissues, bronchial exudates, pleural and pericardial fluids, and exudates in the sinuses and bony spaces of the head when these were found diseased. Thin fluids were inoculated into dextrose broth while thicker exudates were streaked out on plain blood agar. All of the latter and some of the former were controlled by making direct smears, stained according to Gram's method and counterstained with a dilute aqueous fuchsin solution.

During the epidemic there were 198 postmortem examinations, and at each cultures of the diseased tissues and fluids were made as stated. Many times, and this is particularly true in the early part of the epidemic, blood-agar plate cultures from the diseased lungs contained purely fine, moist, transparent, slightly umbilicated, green colonies of gram-positive, lancet-shaped diplococci as such or in short chains. The bacillus of influenza was never found in pure culture and with other organisms in only six lung cultures. For convenience this gram-positive lancet-shaped diplococcus is called a pneumococcus for reasons to be presented later.

As to the incidence of the pneumococcus, the hemolytic streptococcus, and the bacillus of influenza, the first occurred in 144 lung cultures, the second in 58, the third in 6. These results were from a series of 198 postmortem examinations, 26 lung cultures of which were unsatisfactory, and in 5 of which no lung cultures were made. The following table briefly summarizes the results.

Pure pneumococcus	89
Pneumococcus and hemolytic streptococcus	34
Pneumococcus and nonhemolytic streptococcus	13
Pneumococcus, hemolytic, and nonhemolytic streptococci	3
Pneumococcus, influenza bacillus, and nonhemolytic streptococcus	2
Pneumococcus and influenza bacillus	2
Pneumococcus, influenza bacillus, and hemolytic streptococcus	1
Pure hemolytic streptococcus	19
Hemolytic streptococcus and influenza bacillus	1
Nonhemolytic streptococcus	3
Not cultured	5
Unsatisfactory	26
	198

The importance of hemolytic streptococci in diseases of the respiratory passages is well recognized. During the early and middle stages of the epidemic this organism appeared sporadically, so to speak, but in the later stages when complications of pneumonia were observed its presence and frequency in the cultures increased. The incidence of the pneumococcus and the hemolytic streptococcus during the course of the epidemic is detailed graphically in chart 1, and from

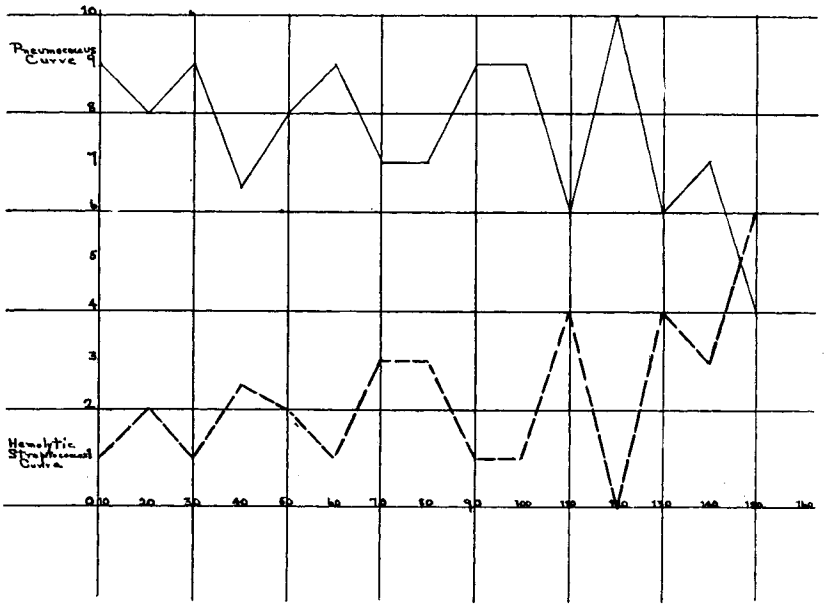

Chart 1.—The incidence curve of pneumococci and hemolytic streptococci in the postmortem bacteriologic studies during the epidemic. The curve was made by arranging in sequence all postmortem examinations with satisfactory bacteriologic studies from the beginning to the close of the epidemic and then in groups of ten noting the frequency of a predominating pneumococcus or hemolytic streptococcus. On the abscissae are plotted postmortem examinations in groups of ten. On the ordinate the number of times the pneumococcus or hemolytic streptococcus was the predominant organism in each group. Pneumococcus ————, Hemolytic Streptococcus — — — —.

this the importance of a careful study from the beginning to the close of the epidemic can be best appreciated. Where only a few chance examinations are made or where the study is limited to some portion of the curve an erroneous impression may be gained regarding the proper importance of the hemolytic streptococcus. Hemolytic streptococci appeared in 58 lung cultures of the 198 postmortem examinations as follows:

Pure hemolytic streptococcus	9
Hemolytic streptococcus and influenza bacillus	1
Hemolytic streptococcus and pneumococcus	19
Hemolytic streptococcus and pneumococcus with pneumococcus in heart blood	10
Hemolytic streptococcus and pneumococcus with hemolytic streptococcus in heart blood	8
Hemolytic streptococcus, pneumococcus, and influenza bacillus with pneumococcus in the heart blood	1
Hemolytic streptococcus with pneumococcus in the heart blood	2
Hemolytic streptococcus both in lungs and heart blood	7
Hemolytic streptococcus in lungs, heart blood and spinal fluid	1
	58
Hemolytic streptococcus in heart blood with pneumococcus in lung	6
Hemolytic streptococcus and pneumococcus in heart blood with pneumococcus in lungs	1

The influenza bacillus was found in cultures with other organisms in 6 postmortem examinations distributed as follows:

Influenza bacillus with many pneumococci	2
Influenza bacillus with pneumococcus and nonhemolytic streptococcus	2
Influenza bacillus with pneumococcus and hemolytic streptococcus	1
Influenza bacillus with hemolytic streptococcus	1
	6

The search for influenza bacilli in smears of the lung exudate and in all cultures was careful and thorough. The possibility of this organism being overgrown by other bacteria on the blood-agar plates is not probable, as surface inoculations uniformly were made so that any organism could be isolated or at least identified.

As control for the surface inoculation of plain blood agar in recovering the influenza bacillus from lung and other exudates as practiced during the epidemic, a large number of throat cultures were made after the epidemic comparing the blood agar surface inoculations with similar surface inoculations made on mediums especially favorable to the growth of the influenza bacillus. The tests were made by collecting posterior pharyngeal secretions on cotton applicators and inoculating in succession with the same cotton applicator the surface of a plain blood-agar plate, an Avery's selective medium plate,[1] and a "chocolate" medium plate. The latter medium was made by drawing horse blood into flasks containing sterile sodium citrate solution to make 1.5% final concentration. After the corpuscles had settled, the plasma was removed and sterile distilled water added up to the original volume of blood. Beef infusion agar with a reaction of plus 0.2% is heated to 90 C., and while at this temperature the laked blood is added up to 1-2%.

In these examinations the influenza bacillus was found as frequently on the blood-agar plates as on the other mediums.

[1] Jour. A. M. A., 1918, 71, p. 2050.

Cultures of the heart blood removed at the postmortem examination yielded most frequently a pneumococcus. The results obtained in 198 postmortem examinations are briefly:

Pneumococcus	53
Hemolytic streptococcus	23
Pneumococcus and hemolytic streptococcus	1
Nonhemolytic streptococcus	2
Negative	109
Not taken	10
	198

Cultures of a limited number of spinal fluids removed at the postmortem examinations including three in which the leptomeninges were clouded by a thick yellow exudate accomplished the isolation of pneumococci in pure culture in eight, and bacterial examination of fluid removed by spinal puncture from patients in the hospital demonstrated pure culture of pneumococci in three others.

TABLE 6

PNEUMOCOCCI FROM BLOOD CULTURES DURING LIFE

Culture	Inulin Fermentation	Capsule		Bile Solubility	Amount of 24-Hour Blood-Agar Slant Injected into White Mouse	Dose Fatal in Hours	Type	Organism Recovered Pure from Mouse Heart Blood
		On Blood Agar	In Heart Blood of Mouse					
602	+	—	+	+	0.0231	18	II	+
605	+	—	+	+	0.0231	22	II	+
610	+	—	+	+	0.0231	18	IV	+
611	+	—	+	+	0.0231	30	IV	+
613	+	—	+	+	0.0231	18	IV	+
624	+	—	+	+	0.0231	18	IV	+
628	+	—	+	+	0.0231	14	II	+
631	+	—	+	+	0.0231	18	II	+
633	+	—	+	+	0.0231	18	IV	+
637	+	—	+	+	0.0231	15	II	+
640	+	—	+	+	0.0231	22	IV	+
643	+	—	+	+	0.0231	18	IV	+
644	+	—	+	+	0.0231	30	II	+
646	+	—	+	+	0.0231	18	II	+
658	+	—	+	+	0.0231	44	IV	+

+ positive; — negative.

Similarly, pure cultures of pneumococci were isolated from four pleural fluids removed postmortem and fluid taken from the pericardial sac at five other necropsies contained organisms morphologically identical in pure culture.

During the epidemic the left knee of a patient in the hospital became swollen and painful. Aspiration disclosed the presence of a

thick yellow exudate which culturally contained purely pneumococci. A postmortem examination later disclosed the presence of an extensive atypical suppurative interstitial pneumonia, culture of the lung tissue containing organisms identical with those recovered from the exudate in the knee joint. The exudate recovered from a gluteal abscess in another patient convalescent from pneumonia contained a pure culture of pneumococci.

In order to determine whether the gram-positive diplococcus isolated from the heart blood, lungs and other diseased tissues of the body was a pneumococcus according to recognized tests, the pure strains recovered were tested for their cultural characteristics on plain blood agar and in Hiss inulin serum water, for their tinctorial reaction

TABLE 7

PNEUMOCOCCI FROM ACCESSORY SINUSES AND EYE

Culture	Inulin Fermentation	Capsule in Heart Blood	Bile Solubility	Amount of 24-Hour Blood-Agar Slant Injected into White Mouse	Dose Fatal in Hours	Type	Organism Recovered Pure from Mouse Heart Blood	Source of Culture
2022	+	+	+	0.00925	36	IV	+	Conjunctiva
2023	+	+	+	0.00925	28	IV	+	Ear
2029	+	+	+	0.00925	40	II	+	Ear
2030	+	+	+	0.00925	18	IV	+	Antrum of Highmore
2056	+	+	+	0.00925	32	IV	+	Mastoid
2080	+	+	+	0.00925	12	IV	+	Rt. frontal, postmortem
2084	+	+	+	0.00925	30	IV	+	Sphenoid sinus, postm.
2087	+	+	+	0.00925	30	II	+	Mastoid

+ positive; — negative.

and morphology by staining according to Gram's method, and for the presence of a capsule by staining according to the Hiss technic. Measured fractional quantities of a 24-hour blood-agar slant were inoculated intraperitoneally into white mice to determine (1) an approximation of the virulence the various strains might have, and (2) to test further the morphology and type of pneumococcus in the mouse. The approximate virulence for white mice, the presence of a capsule, the reaction in inulin, the bile solubility and the type are given in table 2 for a few strains recovered from the heart blood post mortem. The same features are given for a few isolated from the lungs, from various sources, and from spinal fluids in tables 3, 4 and 5, respectively. Without exception, practically all strains not listed reacted culturally the same and appeared identical morphologically.

BLOOD CULTURE DURING LIFE

Having found a pneumococcus in the heart blood at postmortem examination of patients dying with pneumonia during the epidemic, the next step was to attempt its isolation from the blood stream of patients in the hospital. The usual inoculation of a few cubic centimeters of whole blood into broth or other medium did not seem as good a method as that of laking larger quantities of blood and inoculating the centrifugalized sediment into a suitable medium. The method therefore used is as follows: About 10 c c of blood was drawn asceptically into ½ c c of 15% sodium citrate in a large sterile centrifuge tube and thoroughly mixed. To this 4 or 5 volumes of sterile distilled water were added to lake the red corpuscles, and the liquid then centrifuged in a large centrifuge at about 2,000-2,500 r. p. m. for 20-30 minutes. The supernatant liquid was drawn off with a sterile pipet, and the sediment inoculated into melted blood agar and plated in sterile Petri dishes. After 24-36 hours' incubation, the plates were examined for colonies of bacteria.

During the first few days of the epidemic, cultures were made from the blood of patients with pneumonia, and of the number taken, seventeen contained small green colonies of pneumococci. At the beginning, the total number of cultures on epidemic patients was not separated from others taken in the hospital, so that in this series, the percentage of positive results cannot be determined accurately. These first attempts, however, demonstrated clearly that invasion of the blood stream by a pneumococcus was present, and at once a systematic investigation was started. Using the method outlined, and without more than one attempt in all but 3 or 4, blood cultures were made on 90 patients in the hospital wards. Of this number 45 were positive, the colonies on the blood-agar plates without a single exception being small, moist, surrounded by a distinct green halo and containing organisms morphologically pneumococci.

The total number of strains obtained pure by blood cultures is 62, and based on the series of 90 blood cultures taken, in which 45 proved positive, the organism was isolated in 50% of the patients tested. That this does not represent the highest percentage possible is understood by the statement that with the exception of three or four patients, only one culture was made with each patient. Of all the positive blood cultures obtained from patients in the hospital with the epidemic disease not one contained organisms other than the pneumococcus.

Additional cultural and morphological data for a few of these strains are contained in table 8.

TABLE 8

PNEUMOCOCCI IN PLEURAL EXUDATES

Culture	Inulin Fermentation	Capsule in Heart Blood	Bile Solubility	Amount of 24-Hour Blood-Agar Slant Injected into White Mouse	Dose Fatal in Hours	Type	Organism Recovered Pure from Mouse Heart Blood
2024	+	+	+	0.00925	36	II	+
2028	+	+	+	0.00925	28	IV	+
2034	+	+	+	0.00925	24	II	+
2046	+	+	+	0.00925	17	IV	+
2048	+	+	+	0.00925	15	II	+
2051	+	+	+	0.00925	32	I	+
2054	+	+	+	0.00925	17	II	+
2060	+	+	+	0.00925	15	IV	+
2063	+	+	+	0.00925	15	I	+
2066	+	+	+	0.00925	12	IV	+
2069	+	+	+	0.00925	12	II	+
2085	+	+	+	0.00925	30	II	+

+ positive.

CULTURES OF EXUDATES RECOVERED FROM SINUSES AND BONY SPACES OF THE HEAD

Invasion of the bony spaces of the head is not uncommon in severe infections of the nasopharynx. It was not surprising, therefore, to find such complications in patients during the epidemic. In such, the nasopharynx or upper respiratory passages had been especially if not solely involved. Acute suppurative otitis media was the most common severe upper respiratory complication. Many of these diseased middle ears were drained without bacteriologic examination so that the reports of such with bacteriologic studies are few. Others examined bacteriologically several days after artificial drainage contained such a variety of organisms that the initial invader could not be determined. The exudate escaping with paracentesis of the ear-drum in three patients was cultured on plain blood-agar plates, and the growth after 24 hours' incubation was purely fine green colonies of pneumococci. Other organisms were not present.

In two patients acute suppurative mastoiditis occurred and from the exudate pure cultures of the same organism were recovered. In two other patients involvement of the antrum of Highmore followed the nasopharyngeal infection and from the exudate a pure culture of pneumococci was isolated. And cultures of exudates discovered in the bony sinuses of the head at the postmortem examinations again gave

the organism in pure culture. The conjunctiva in one patient became infected and a pure culture of pneumococci was isolated from the exudate. The results of morphological, cultural and virulence tests of the cultures isolated from the sinuses of the ear and the eye are given in table 7.

CULTURES OF PLEURAL EXUDATES

The collection of a purulent exudate in the chest as a complication of pneumonia is common. The acute stages of pneumonia in a large number of patients admitted during the epidemic was followed or complicated by empyema. This is not the place to discuss at length empyema following closely in the wake of the epidemic proper, but it does seem important to give here the results of cultures of the fluid aspirated from the chest of patients in whom a purulent exudate formed. The exudate was removed aseptically with needle and syringe, and cultured promptly on plain blood-agar plates. The aspirated fluid from 74 empyemas was examined with the following results:

Pure pneumococcus	46
Pneumococcus and hemolytic streptococcus	5
Pure hemolytic streptococcus	22
Pure nonhemolytic streptococcus	1
Total	74

This table shows that a pneumococcus was recovered in pure culture in 62% of the fluids examined. These strains of pneumococci, too, were identified more closely by further study, the results of which are shown in table 8.

VIRULENCE EXPERIMENTS

During the sudden rush of work coming necessarily with an epidemic, certain general methods must be followed in order to gather as much material as possible, to be studied later. From many sources pure strains of pneumococci were isolated and kept on artificial mediums until their cultural, morphologic, and virulence properties could be systematically determined. Realizing that the virulence of these organisms might diminish with cultivation on artificial mediums the determination of virulence was taken up with least possible delay. With some cultures, however, the determination necessarily was delayed until 4 or 5 weeks after their first isolation.

The white mouse was chosen for this purpose, as both virulence determination and typing of the pneumococcus could be done in one animal. Rabbits and guinea-pigs were used also. In order to approximate a standard unit for inoculation, all transfer cultures were inoculated on plain blood agar slanted in culture tubes of 16 by 150 mm. dimension, approximately the same surface area being inoculated with the same quantity each time. The growth after 24 hours' incubation was suspended in measured quantities of normal salt solution, the surface of the agar being brushed off with a camel's hair brush. Further standardization was accomplished by establishing a unit loop made of medium sized platinum wire. The number of loops in 1 c c of salt solution was found to be 108. Taking up the 24 hours' growth of bacteria on a blood-agar slant in a definite number of cubic centimeters of salt solution, and knowing the number of loops contained in one c c the amount of a 24-hour blood-agar slant culture inoculated can be expressed in a decimal fraction.

All the strains isolated from the heart blood, from the lungs, and from various other foci of the body postmortem, from the circulating blood, the middle ear, the maxillary sinuses, the pleural cavity, and from other places were tested for their virulence in white mice.

In order to carry out virulence tests on such a large number of cultures it was necessary to determine approximately the virulence of a few strains in white mice, and having determined a minimal dose in a small group to try this same amount in a large number. For the strains isolated from the blood stream and for those isolated from the heart blood postmortem 0.0231 of 24-hour blood-agar culture killed constantly, some of the cultures causing death sooner than others. The number of hours required to kill a white mouse of average size is given for some of the heart blood cultures in chart 4, and for certain blood cultures in chart 8.

When the virulence of the strains isolated from the lungs postmortem and of those isolated from various exudates obtained during life was examined, it was found to exceed that determined for the heart blood and the blood culture strains. Consequently the dosage was diminished at first to 0.0154 of 24-hour blood-agar slant culture, and when this was found to be rapidly fatal it was diminished to 0.00925 of a culture, then to 0.00555, and finally as low as 0.00185. The smallest dose tested in a few cultures was found to be fatal to white mice in from 30-36 hours. The amount of 24-hour blood-agar culture inoculated into a white mouse and the time required to kill is

given for some of the lung cultures in table 3, for those recovered from the spinal fluid postmortem and during life in table 5, and for those isolated from pleural and the pericardial fluids and from embolic foci postmortem and during life in table 4, for those from pleural exudates in table 8, for those from infected sinuses of the head, mastoid, and eye in table 7, and for those from the throat in table 3.

In all of these virulence experiments the organism practically without exception was recovered from the heart blood of the mouse in pure culture. The peritoneal exudate was used in determining the type of pneumococcus with the antipneumococcus agglutinating serums.

To determine the virulence of some of the strains in rabbits and guinea-pigs further tests were made as follows:

1.—Rabbit, 1,625 gm. injected intravenously with 1-18 hour blood-agar slant of blood culture No. 655. Dead after 16 hours. Pure culture of organism recovered from heart blood and peritoneal exudate.

2.—Guinea-pig, 285 gm., injected intraperitoneally with 1-22 hour blood-agar slant culture of blood culture No. 655. Dead after 13 hours. Pure culture of organism recovered from the heart blood and peritoneal exudate.

3.—0.25 of 1-24 blood-agar slant culture of designated strains was inoculated intraperitoneally as follows: Guinea-pig, 345 gm., blood culture No. 606, dead after 60 hours. Guinea-pig, 297 gm., blood culture No. 616, dead after 39 hours. Guinea-pig, 325 gm., blood culture No. 651, dead after 39 hours. Guinea-pig, 325 gm., blood culture No. 658, dead after 47 hours. Guinea-pig, 288 gm., blood culture No. 604, lived. Guinea-pig, 390 gm., blood culture No. 621, lived. Guinea-pig, 326 gm., blood culture No. 608, lived. Guinea-pig, 277 gm., blood culture No. 611, lived. Guinea-pig, 334 gm., blood culture No. 639, lived. Guinea-pig, 298 gm., blood culture No. 660, lived. Organism recovered in pure culture from the heart blood and peritoneal exudate of all the animals that died.

4. 1-18 hour blood-agar slant culture of heart blood culture No. 1000 washed off in 4 c c of salt solution. Guinea-pig, 245 gm., received ½ c c of bacterial suspension intraperitoneally. Lived. Guinea-pig, 345 gm., received 1 c c of bacterial suspension intraperitoneally. Lived. Guinea-pig, 330 gm., received 2½ c c of bacterial suspension intraperitoneally. Dead after 45 hours. Organism recovered in pure culture in the heart blood and in the peritoneal exudate.

5.—1-18 hour blood-agar slant culture of heart blood culture No. 1004 washed off in 4 c c of salt solution. Guinea-pig, 230 gm., received ½ c c of bacterial suspension intraperitoneally, dead after 17 hours. Guinea-pig, 234 gm., received 1 c c of bacterial suspension intraperitoneally, dead after 19 hours. Guinea-pig, 255 gm., received 2½ c c of bacterial suspension intraperitoneally, dead after 20 hours. Pure culture of organism isolated from the heart blood and peritoneal exudate of each animal.

6.—Inoculated 0.5 of 1-24 hour blood-agar slant culture of designated strains intraperitoneally into guinea-pigs as follows: Guinea-pig, 308 gm., heart blood culture No. 1036, dead after 44½ hours. Guinea-pig, 325 gm., heart blood culture No. 1040, dead after 10 hours. Guinea-pig, 290 gm., heart blood culture No. 1036, lived. Guinea-pig, 269 gm., heart blood culture No. 1037, dead after 68 hours. Guinea-pig, 332 gm., heart blood culture No. 1033, dead after 6 days. Organism recovered in pure culture from heart blood and peritoneal exudate.

7.—1-24 hour blood-agar slant culture of lung culture No. 331 suspended in 5 c c of normal salt solution. Guinea-pig, 303 gm., 0.1 culture intraperitoneally, dead after 47 hours. Guinea-pig, 244 gm., 0.2 culture intraperitoneally, dead after 19 hours. Guinea-pig, 264 gm., 0.3 culture intraperitoneally, dead after 33 hours. Guinea-pig, 265 gm., 0.4 culture intraperitoneally, dead after 25½ hours. Organism recovered in pure culture from heart blood and peritoneal exudate of all animals.

8.—1-24 hour blood-agar slant culture of lung culture No. 350 suspended in 5 c c of normal salt solution. Rabbit, 1,320 gm., 0.1 culture intravenously, dead after 31 hours. Rabbit, 1,580 gm., 0.2 culture intravenously, dead after 37 hours. Rabbit, 1600 gm., 0.3 culture intravenously, dead after 20 hours. Rabbit, 1590 gm., 0.4 culture intravenously, dead after 15 hours. Organism recovered in pure culture from heart blood of each animal.

9.—Rabbit, 2222 gm., 0.1-24 hour culture of lung culture No. 347 intravenously, dead after 6¾ days. Organism recovered pure from the heart blood. Rabbit, 2,360 gm., 0.1-24 hour culture of lung culture No. 306 intravenously, lived, lost 200 gm. in 5 days. Rabbit, 2,165 gm., 0.1-24 hour culture of lung culture No. 340 intravenously, lived, lost 370 gm. in 10 days.

10.—Guinea-pig, 274 gm., 0.1-24 hour culture of lung culture No. 347 intraperitoneally, dead after 60 hours. Guinea-pig, 290 gm., 0.1-24 hour culture of lung culture No. 306, intraperitoneally, dead after 60 hours. Guinea-pig, 297 gm., 0.1-24 hour culture of lung culture No. 340 intraperitoneally, dead after 60 hours. Pure cultures of the organism were isolated from heart blood and peritoneal cavities of all the animals.

11.—Quantitative virulence experiment in white mice. Lung culture No. 347. White mouse, 0.00925 24 hour culture, dead after 17 hours. White mouse, 0.00925 24 hour culture, dead after 19½ hours. White mouse, 0.000925 24 hour culture, dead after 19 hours. White mouse, 0.000925 24 hour culture, dead after 22 hours. White mouse, 0.0000925 24 hour culture, dead after 22 hours. White mouse, 0.0000925 24 hour culture, dead after 28 hours. Pure cultures of the organism were isolated from heart blood and peritoneal cavities of all the animals.

12.—Quantitative virulence experiment in white mice. Lung culture No. 306. White mouse, 0.00925 24 hour culture, dead after 13½ hours. White mouse, 0.00925 24 hour culture, dead after 13½ hours. White mouse, 0.000925 24 hour culture, dead after 14½ hours. White mouse, 0.000925 24 hour culture, dead after 14½ hours. White mouse, 0.0000925 24 hour culture, dead after 14½ hours. White mouse, 0.0000925 24 hour culture, dead after 15 hours. Pure cultures of the organism were isolated from heart blood and peritoneal cavities of all the animals.

13.—Quantitative virulence experiment in white mice. Lung culture No. 340. White mouse, 0.00925, 24 hour culture, dead after 14½ hours. White mouse, 0.00925, 24 hour culture, dead after 27½ hours. White mouse, 0.000925, 24 hour culture, dead after 26½ hours. White mouse, 0.000925, 24 hour culture, dead after 26½ hours. White mouse, 0.0000925, 24 hour culture, dead after 14½ hours. White mouse, 0.0000925, 24 hour culture, dead after 27½ hours. Pure cultures of the organism isolated from heart blood and peritoneal cavities of all the animals.

14.—0.1 of 1-24 hour blood agar slant culture of designated lung strains was inoculated intraperitoneally as follows: Guinea-pig, 390 gm. lung culture No. 310, lived, lost 35 grams in 2 days. Guinea-pig, 293 gm., lung culture No 305, dead after 68 hours. Guinea-pig, 263 gm., lung culture No. 344, dead after 6 days. Guinea-pig, 413 gm., lung culture No. 358, dead after 9 days. Guinea-pig, 389 gm., lung culture No. 365, lived, no effect. Guinea-pig, 359 gm., lung culture No. 369, lived, lost 93 grams in 8 days. Guinea-pig, 274 gm., lung culture No. 380, lost 20 gm. in 6 days. Guinea-pig, 376 gm., lung culture No. 376, dead after 52 hours. Guinea-pig, 396 gm., lung culture No. 398, lived, lost 73 grams in 8 days. Guinea-pig, 365 gm., lung culture No. 406, dead after 50 hours. Organism recovered in pure culture from the heart blood and peritoneal exudate of all the animals that died.

15.—0.1 of 1-24 hour blood-agar slant culture of the following designated strains injected intraperitoneally as follows: Guinea-pig, 340 gm., spinal fluid culture No. 3042, dead after 28½ hours. Guinea-pig, 362 gm., spinal fluid culture No. 2083, lived, lost 51 grams in 4 days. Guinea-pig, 430 gm., pleural fluid culture No. 2070, lived, lost 96 grams in 4 days. Guinea-pig, 433 gm., mastoid culture No. 2056, lived, lost 51 grams in 4 days. Guinea-pig, 383 gm., mastoid culture No. 2087, dead after 14 hours. Guinea-pig, 324 gm., pleural fluid culture No. 2089, dead after 24 hours. Guinea-pig, 355 gm., pleural fluid culture No. 2051, lost 43 gm. in 4 days. Pure culture of organism recovered from peritoneal cavity of 11 the animals that died and from the heart blood of these same animals excepting guinea-pig (No. 2087).

16.—0.1 of 1-24 hour blood-agar slant culture of designated strains injected intravenously as follows: Rabbit, 1549 gm., spinal fluid culture No. 2042, lived and lost 120 gm. in 4 days. Rabbit, 1751 gm., spinal fluid culture No. 2083, lived and lost no weight. Rabbit, 1439 gm., mastoid culture No. 2056, lived and lost 140 gm. in 2 days. Rabbit, 1569 gm., mastoid culture No. 2087, lived and lost 150 gm. in 2 days. Rabbit, 1792 gm., pleural fluid culture No. 2070, dead after 24 hours.

These virulence tests have demonstrated a highly lethal property for practically all cultures in animals, a small part of a 24-hour blood-agar slant culture being regularly fatal.

Throat cultures have proven to be the least virulent. The detection of virulent strains of pneumococci is impossible simply by judging from the appearance of the colony, and in transfering many colonies nonvirulent strains may be isolated as readily as the virulent, depending, of course, on the frequency of one or the other, and the element of chance. Our throat cultures, further, were grown on artificial mediums for some time before virulence tests could be made. However, it is safe to say that the upper respiratory passages were invaded by virulent pneumococci inasmuch as a number of definitely virulent strains were isolated from the throat, while organisms quite as virulent as those recovered from the lungs, were isolated from the sinuses of the head and from the middle ear.

The inoculations in white mice indicate high virulence. The smallest dilution, representing 0.0000925 of a 24-hour blood-agar slant culture, killed in a little more than 12-24 hours. In order to determine how many organisms this dilution represents three 24-hour blood-agar slant cultures were taken up, respectively, in 5 c c of normal salt solution and dilutions made as before:

(*a*) Five loops of bacterial suspension in 1 c c sterile distilled water — 5/540 or 0.00925 per c c.

(*b*) 0.5 of (*a*) in 4.5 c c sterile distilled water — 5/5,400 or 0.000925 per c c.

(*c*) 0.5 c c of (*b*) in 4.5 c c distilled water.

One c c of dilution (c) representing 0.0000924 of a culture was centrifuged in a clean sterile centrifuge tube at 2,000 to 2,500 r. p. m. for 1 hour. The supernatant liquid was removed with a capillary pipet and the last drops containing the bacteria transferred to a slide, allowed to dry, fixed, stained, and the pneumococci counted. The average count of these three estimations was 200 organisms, there being a variation of 35 between the maximum and the minimum counts. It seemed theoretically possible at least, from these determinations to increase the final dilution given above by 10 and have a suspension containing roughly 20 organisms, to the c c., or to increase that same dilution by 100 and have a suspension containing about 2 organisms in a c c. Accordingly, 24-hour blood-agar slant growths of lung cultures 306 and 347 were prepared. The growth of each one was taken up in 5 c c of normal salt solution and dilutions then made with sterile distilled water as follows:

(a) Fifteen loops of bacterial suspension in 3 c c sterile distilled water — 5/540 or 0.00925 culture per c c.

(b) 0.5 c c of (a) in 4.5 c c sterile distilled water — 5/5400 or 0.000925 per c c.

(c) 0.5 c c of (b) in 4.5 c c sterile distilled water — 5/54000 or 0.0000925 per c c.

(d) 0.5 c c (c) in 4.5 c c sterile distilled water —5/540000 or 0.00000925 culture per c c.

(e) 0.5 c c of (d) in 4.5 c c sterile distilled water —5/5400000 or 0.000000925 culture per c c.

Considered on the basis of the estimations which gave approximately 200 organisms in 0.0000925 cultures as represented by dilution (c), then dilution (d) would contain about 20 organisms per c c, and dilution (e) only a few; 10 white mice were selected for each series of culture dilutions, and in groups of two they were inoculated as follows:

Lung culture No. 306.—White mouse, 0.00925 24 hour culture, dead after 12 hours. White mouse, 0.00925 24 hour culture, dead after 12 hours. White mouse, 0.000925 24 hour culture, dead after 14 hours. White mouse, 0.000925 24 hour culture, dead after 15 hours. White mouse, 0.0000925 24 hour culture, dead after 32 hours. White mouse, 0.0000925 24 hour culture, dead after 24 hours. White mouse, 0.00000925 24 hour culture, dead after 30 hours. White mouse, 0.00000925 24 hour culture, lived. White mouse, 0.000000925 24 hour culture, lived. White mouse, $0.000000925 24 hour culture, lived.

Lung culture No. 347.—White mouse, 0.00925 24 hour culture, dead after 12 hours. White mouse, 0.00925 24 hour culture, dead after 12 hours. White mouse, 0.000925 24 hour culture, dead after 12 hours. White mouse, 0.000925 24 hour culture, dead after 15 hours. White mouse, 0.0000925 24 hour culture, dead after 12 hours. White mouse, 0.0000925 24 hour culture, dead after 12 hours. White mouse, 0.00000925 24 hour culture, dead after 12 hours. White mouse, 0.00000925 24 hour culture, dead after 15 hours. White mouse, 0.000000925 24 hour culture, dead after 17 hours. White mouse, 0.000000925 hour culture, dead after 17 hours. Pure cultures of the organism were isolated from heart blood and peritoneal cavities of all the animals.

After the inoculation of each dilution the glass syringe and needle used were placed in boiling water so that there could be no transfer of organisms in succeeding inoculations, and three control mice after each had received 1 c c of sterile distilled water intraperitoneally were placed in the cage with the inoculated mice. To control further the amount of bacterial suspension

inoculated, 1 c c of dilution (d) of each culture representing 0.00000925 culture was centrifuged in a clean centrifuge tube for 1 hour at 2,000 r. p. m., and in the stained sediment no pneumococci was found. Where only a few bacteria are contained in a liquid it is quite within the limits of technical error to fail in finding them, but at any rate the experiment indicates that the number of organisms suspended in the tested dilution was very small. To find that all of the mice inoculated with dilutions of culture 347 were dead within 17 hours is rather startling, and were the experiment not controlled carefully one might think that the results were falsified in some way. However, the results obtained with culture 306 as detailed, support the results obtained with culture 347, and pure cultures of pneumococci were isolated from the heart blood of all the animals that died. All of the control mice lived.

Following the lung cultures in the degree of virulence are the strains recovered from the heart blood at postmortem and from blood cultures during life. Why the strains isolated from the lungs and other sources of the body should possess the greatest virulence is not clear.

TABLE 9

THE RESULTS OF TYPE DETERMINATIONS OF PNEUMOCOCCI

Source	No. of Cultures Tested	Type I	Type II	Type III	Type IV
Throat	8	..	3	..	5
Lungs at postmortem	201	12	109	2	78
Heart blood	67	5	45	..	17
Spinal fluid	12	3	7	..	2
Miscellaneous	13	3	3	..	7
Blood cultures	57	1	35	..	21
Exudates from:					
I Sinuses of head	8	..	2	..	6
II Chest	38	6	15	..	17
Total	404	30	219	2	153
Percentages	.....	7.42%	54.20%	0.49%	37.88%

An organism has been described which, according to standard methods of recognition, is a pneumococcus. All the investigations of the epidemic demonstrate clearly that if the organism isolated from the sources mentioned be considered a pneumococcus, it must possess qualities which make it distinctive from other pneumococci, both in the power of invading tissues and in the lesion it produces in the host. All experimental work with this pneumococcus supports the conclusion that the strains of pneumococci recovered during the epidemic possess a high virulence. In this respect these organisms demonstrate a feature which makes them distinctive from other pneumococci. Such distinctive quality further explains why these pneumococci were able to produce an epidemic disease.

The results of the type determinations are given in table 9.

CONTROL INVESTIGATIONS

In order to have comparative data for the many throat examinations made on patients during the epidemic, throat cultures were taken on plain blood-agar plates from 50 of the prisoners. This group of men had been in the camp since last spring (1918), and not one of them had been ill with the

TABLE 10

THROAT CULTURES FROM PRISONERS—ORGANISMS OTHER THAN PNEUMOCOCCUS

Cultures	Organisms Found
1	Streptococci, nonhemolytic
2	Streptococci, nonhemolytic, staphylococcus
3	Streptococci, hemolytic
10	Streptococci, nonhemolytic
12	Streptococci, hemolytic
13	Streptococci, nonhemolytic, staphylococcus
14	Streptococci, hemolytic, staphylococcus
17	Streptococci, nonhemolytic
22	Streptococci, hemolytic
26	Streptococci, nonhemolytic, staphylococcus and Micrococcus catarrhalis
27	Streptococci, hemolytic, staphylococcus
28	Streptococci, hemolytic
29	Streptococci, nonhemolytic, staphylococcus
30	Streptococci, nonhemolytic, staphylococcus and Micrococcus catarrhalis
31	Streptococci, hemolytic, staphylococcus
32	Streptococci, nonhemolytic, staphylococcus
33	Streptococci, hemolytic, staphylococcus
36	Streptococci, hemolytic, staphylococcus
37	Streptococci, nonhemolytic, staphylococcus
38	Streptococci, hemolytic, staphylococcus
42	Streptococci, hemolytic, staphylococcus
43	Streptococci, hemolytic, staphylococcus
44	Streptococci, hemolytic, staphylococcus
47	Streptococci, hemolytic, staphylococcus

TABLE 11

THROAT CULTURES FROM PRISONERS — PROPERTIES OF PNEUMOCOCCI ISOLATED

Culture	Inulin Fermentation	Capsule		Bile Solubility	Amount of 24-Hour Blood-Agar Slant Injected into White Mouse	Dose Fatal in Hours	Type	Organism Recovered Pure from Mouse Heart Blood
		On Blood Agar	In Heart Blood of Mouse					
4	+	—	—	..	0.25	—		
6	+	—	—	..	0.25	+		
7	—	—	—	+	1.0	12	IV	—
8	+	—	—	..	0.5	—		
9	—	—	—	..	0.5	—		
15	—	—	—	+	1.0	12	IV	+
11	—	—	—	..	0.5	—		
16	+	—	—	..	0.5	—		
18	—	—	—	..	0.5	—		
19	+	—	—	..	0.25	—		
20	+	—	—	..	0.25	—		
21	+	—	—	..	0.5	—		
23	—	—	—	+	1.0	12	IV	—
24	+	—	—	..	0.5	—		
25	+	—	—	+	0.5	31	IV	+
34	—	+	+	+	1.0	12	II	+
35	+	—	—	..	0.25	—		
39	+	—	—	..	0.25	—		
40	—	—	+	..	0.5	14	IV	—
41	+	—	—	+	0.5	14	IV	—
45	—	—	—	..	0.5	—		
48	+	—	—	+	0.5	14	IV	—
49	—	—	—	..	0.5	—		
50	—	—	—	..	0.5	—		

+ positive; — negative.

epidemic disease. The cultures were made with sterile cotton applicators, the secretions collected from the posterior pharynx being inoculated in surface smears on plain blood agar plates.

Table 10 records the bacterial flora in the 24 throat cultures in which pneumococci were not found. Chart 12 details certain morphological, cultural and virulence tests of a pneumococci isolated in 25 other throat cultures. One of the throat cultures was unsatisfactory. White mice were inoculated intraperitoneally with one-quarter, one-half, and entire 24-hour blood-agar slant cultures of the isolated strains of pneumococci. Eight of these strains of which one-half and entire 24-hour cultures had been inoculated were fatal to the mice. Therefore it was necessary to try a smaller quantity One-quarter of a 24-hour growth of these strains was then inoculated. Six of the eight mice died, but in only three was the organism recovered from the heart blood. Given to guinea-pigs in the same quantity, these three strains had no effect.

The result of this survey indicates that with the exception of three strains of pneumococci, the pneumococci recovered in throat cultures of the prisoners possessed no unusual pathogenic property.

SUMMARY

An epidemic of unusual virulence swept with great rapidity through several organizations in Camp Grant between Sept. 21, 1918, and Oct. 18, 1918 (approximately). During this time 9,037 patients were admitted to the base hospital, representing about one-fourth the strength of the camp, and of them, 26% developed pneumonia. About 11% of the total admissions, or 43% of the total pneumonia patients died.

From the throat cultures of the early admissions pneumococci were recovered with remarkable constancy, influenza bacilli were found exceptionally.

Postmortem examination of patients dying during the epidemic demonstrated regularly a massive bronchopneumonia.

In cultures of the diseased lungs, heart blood, exudates, fluids, and other diseased foci of the body, pneumococci were the predominating organisms.

Blood cultures of 90 patients in the hospital with pneumonia of the epidemic disease were positive in 50%, pneumococci being the only organisms recovered.

Pneumococci in pure cultures were recovered from infected sinuses of the head, the middle ear, conjunctival and empyema exudates.

Inoculation of animals with pneumococci isolated during the epidemic demonstrated a high virulence of these organisms.

Control throat cultures made of prisoners in the camp escaping the epidemic disease contained practically no pathogenic organisms.