

THE COMPARATIVE VIRULENCE OF THE PNEUMOCOCCUS IN THE SPUTUM OF LOBAR PNEUMONIA AT VARIOUS STAGES OF THE DISEASE, WITH SPECIAL REFERENCE TO CRISIS.*

JOHN ARTHUR LUETSCHER.

(*From the Medical Clinic, The Johns Hopkins Hospital, Baltimore, Md.*)

There is no acute infection in which the clinical manifestations are more remarkable than those in pneumonia, and of these manifestations a typical crisis is the most striking. With the abrupt drop of temperature, pulse, and respiration, the patient passes from a "state of extreme hazard and distress to one of safety and comfort"¹ without any demonstrable change in the local condition. In solving the problem as to what happens at the time of crisis to produce this phenomenon, we must consider not only the methods of defense which the body adopts to get rid of the infection, but also the invading parasite. With the exception of a slight increase in agglutinins, the only difference so far determined between normal serum and the serum of immunized animals is in the presence of bacteriotropic substances which cause phagocytosis of virulent strains of pneumococci, while normal serum causes little or no phagocytosis.

G. and F. Klemperer, Neufeld, and others have also demonstrated protective substances in the serum of patients convalescing from pneumonia. In these sera, however, the immunity conferred is relatively slight, and they do not cause phagocytosis of virulent strains.

In the winter of 1909 I repeated the work of Graham on the phagocytability of the pneumococcus isolated from the sputum at various stages of the pneumonic process, using normal human serum and leukocytes. Forty hospital cases were studied. It was then noted that while the pneumococcus isolated before crisis was not phagocyted, the strains isolated at the time of crisis and

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¹ Osler, *Practice of Medicine*.

after crisis were actively phagocyted. This indicated some change in the organism other than a bacteriotropic one. This observation suggested the possibility of a virulence crisis at the time of the temperature crisis, since it had been generally established that the degree of phagocytosis of a given strain was a relative index of its virulence.

This problem has interested many investigators, and I shall attempt to give a rather full historical review because their results have been so vague and conflicting due to the various methods employed, and hence shall lay especial stress on the mode of isolation, amount injected, site of inoculation, weight of animal, and results obtained.

HISTORICAL.

With the discovery of the pneumococcus as the etiological factor of croupous pneumonia, various observers at first attempted to show the relative virulence of the pneumococcus from the various sources, in health and in disease, and later, to establish a virulence curve during the course of lobar pneumonia.

Fränkel isolated the pneumococcus by injecting one loopful of sputum¹ subcutaneously. After establishing the fact that he could demonstrate the pneumococcus for several weeks after crisis, he concludes as follows: "It seems to me that the virulence of the sputum after the crisis is not so high. During the active process, the rabbits died within 24 hours, while after crisis death was delayed several days, often until the sixth day."

Netter asserts that the expectoration during the first weeks after crisis is less virulent than before, that shortly after convalescence the saliva loses all virulence, but later regains its full pathogenicity.

Patella obtained lung juice at different stages of pneumonia by means of a hypodermic syringe. His results agree with those of Netter, that the pneumococcus gradually loses its virulence during the development of the pneumonic process.

Welch does not think that we are justified in laying down such definite laws regarding the loss of virulence as those made by Patella and Netter. He states that he has obtained from pneu-

¹ Personal communication to Dr. Stuetz.

monia at the time of crisis, and during recovery, pneumococci which were as virulent and sometimes more virulent than those procured from earlier stages in the same case.¹

Kruse and Pansini isolated the pneumococcus at various stages of pneumonia by injecting 1 to 4 c.c. of sputum subcutaneously into rabbits. If the animal died with a septicemia, a broth culture was made from the blood; if, however, death occurred late, as a result of a local affection, the organisms were first isolated by the plate method and then transferred to broth. These broth cultures were placed in the thermostat for 24 hours, and 1 to 2 c.c. of the resultant growth were injected subcutaneously. Their conclusion is that the pneumococcus does not suffer a loss of virulence in the course of the pneumonic process. They were also of the opinion that the variation of virulence, determined by the inoculation of the original material from various sources, was probably due to a variation of the number of organisms injected, and also to the fact that many of the bacteria may have been dead.

Eyre and Washbourn isolated the pneumococcus by injecting the sputum into mice. After the death of the animals, cultures were made from the blood on blood agar tubes. A special loop containing about 0.5 gm. was used as a measure for subsequent inoculations into mice. The rusty sputum of two cases of pneumonia and the saliva of one normal case were studied.

Case 1.—Rusty sputum from a case of pneumonia. Two loops of the isolated culture injected subcutaneously into a rabbit were fatal in five days.

Case 2.—Rusty sputum from a case of pneumonia. One loop of the isolated culture injected intraperitoneally into a rabbit was fatal in three days.

Case 3.—Saliva from a healthy individual. One loop injected subcutaneously into a rabbit was fatal in five days.

Stuertz followed the virulence of the pneumococcus in the course of lobar pneumonia by injecting 1 c.c. of sputum subcutaneously into mice weighing 15 to 16 gms. The sputum was collected into sterile dishes, and 1 c.c. was drawn into a hypodermic syringe from different parts of the sputum. He followed

¹ Methods and protocols not published.

20 cases, in 11 of which he constructed charts showing the virulence. In the three cases with crisis, there was a rapid fall of virulence, but not until 24 hours after the drop of the temperature. He therefore concludes that the loss of virulence cannot be the cause of crisis. In the cases with lysis, the fall of virulence was more gradual and more or less parallel with the temperature curve. He also found that extensions of the disease are accompanied by increased virulence, and that we can detect this extension earlier by mice inoculation than by physical signs. Hence he thinks this has a prognostic value, since it puts the physician on his guard at critical moments.

Park and Williams placed a portion of sputum or material to be studied into serum broth at 36° C. for 24 hours. Of this they injected 3 to 4 c.c. subcutaneously into rabbits. Of the culture isolated from the blood at autopsy, and incubated 24 hours in serum broth, 0.1 and 4 c.c. respectively were injected into the ear veins of two rabbits. They also isolated cultures from the original material by means of blood agar plates, and after 24 hours at 36° C., colonies were transferred to blood agar slants and incubated. From these, transfers were made to serum broth, and after 24 hours' growth, tests for virulence were made as above. Isolations by the plate method were found to be distinctly less virulent than those by animal inoculation.

Their results as to the comparative virulence of the pneumococcus obtained from cases of pneumonia and from healthy individuals, with strains isolated by animal inoculation were as follows:

Amount Inoculated	Pneumonia Cases	Healthy Individuals
0.1 c.c.	51 per cent were fatal	31 per cent were fatal
4.0 c.c.	87 " " " "	69 " " " "

Eyre, Leathem, and Washbourn isolated strains of pneumococci by injecting lung juice or saliva subcutaneously or intra-peritoneally into rabbits and transferring the heart's blood at autopsy to blood agar tubes. The resultant growth was tested for virulence.

A) *One case of fatal lobar pneumonia:*

- 1 loop inoculated intraperitoneally was fatal in 3 days. Large number of cocci in blood.
- .001 loop inoculated intraperitoneally was fatal in 4½ days. Fair number of cocci in blood.
- .000,001 loop inoculated intraperitoneally was fatal in 4 days. Very few cocci in blood.

B) *Three cases from saliva:*

1) Saliva from case of carcinoma:

1 loop inoculated subcutaneously killed in 6 days, heart's blood, nil.

.001 " " " " 9 " " "

.000,001 " " " " 8 " " "

2) Normal saliva from a case of occupation paralysis:

With the above doses, the animals died in 11, 6, and 8 days respectively.

3) Normal saliva from a case of interstitial nephritis:

With the above doses, the animals died in 12, 33 days, and 24 hours respectively.

The last was accidentally injected intraperitoneally.

Longcope and Fox isolated three groups of organisms:

A) 16 cultures were obtained from pathological material, such as blood, consolidated lungs, empyema, otitis media, spinal fluid, and endocarditis. They all coagulated inulin and had capsules. In this group they do not state how the organisms were isolated.

B) 35 strains were obtained from healthy individuals by inoculating 2 c.c. of saliva subcutaneously into white mice. At autopsy, cultures were made from blood and from tissues.

a) 19 coagulated inulin and had capsules.

b) 16 did not coagulate inulin or show capsules.

In testing these strains for virulence no definite routine was followed. Some were inoculated subcutaneously, others intraperitoneally. The weight of the rabbits varied from 300 to 2,240 gms. The dose used for inoculation was not constant, but varied from 0.1 c.c. of a broth culture to three tubes of blood agar slants. No minimal lethal dose was determined. The following table was compiled from the results which they obtained in testing the strains of the three groups for their virulence in rabbits:

No. of RABBITS INOCU- LATED	DOSE EMPLOYED	A		B			
				a)		b)	
		Fatal	Not Fatal	Fatal	Not Fatal	Fatal	Not Fatal
1.....	0.1 c.c. 24-hr. broth culture	1
1.....	0.2 c.c. " " " "	1
3.....	0.5 c.c. " " " "	3
6.....	1. c.c. " " " "	4	1	1
9.....	2. c.c. " " " "	3	2	2	..	2
4.....	4. c.c. " " " "	1	2	1
16.....	1 tube 24-hr. growth blood agar	3	4	2	7
4.....	3 tubes " " " "	1	1	2

Strains in group a) were, as a rule, much less virulent than those in group A, and exaltation of virulence was at times difficult.

Strains in group *b*) showed a very slight grade of virulence; in large doses they did sometimes kill rabbits, but the organisms could rarely be cultivated from the organs at autopsy.

Duval and Lewis isolated their organisms by means of the plate method. Agar plates were used to which were added drops of defibrinated blood by means of a sterile pipette. They tested the virulence of 35 recently isolated cultures obtained in large part from pneumonic lungs. Young rabbits were injected with either 10 c.c. of a 24-hour growth on glucose broth, or with the growth of a 24-hour culture on glucose agar plus rabbit's blood. The injections were usually made into the peritoneum. Only one fatal result followed. They found that when they injected the original material into animals their results fell into two classes. If the original material was in pure culture (as determined by plate method) the animal survived large quantities of material. If the original material was a mixture of bacteria the injection of a small dose usually gave a fatal result in one to four days. They also observed that faulty conclusions were drawn if strains were used which had been isolated by animal inoculations.

Buerger isolated 51 strains of pneumococci from the mouth by means of serum glucose agar plates. The material from which these isolations were made was obtained by means of sterilized swabs, which were rubbed against the pillars, tonsils, and posterior pharyngeal wall. The virulence of these strains was determined by injecting a 24-hour growth of one tube of serum agar slant, suspended in 1 c.c. normal saline solution. Injections were made subcutaneously into the backs of white mice. Strains were only two or three generations removed from the original plates.

His results were as follows:

	Virulent	Avirulent	Percentage	Percentage
38 strains isolated from normal throats.....	30	8	79	21
13 " " " " throats of pneumonic cases.....	10	3	77	23

Jürgens used the same method as Stuertz. He selected cases uncomplicated by such factors as heart disease, nephritis, pregnancy, etc. His conclusions were that there must be other factors besides the virulence of the organism, since the organisms iso-

lated from those getting well often had a higher virulence than the strains isolated from cases proving fatal. He found also, that the virulence usually increases when there is progression of the disease, but claimed that at most it is found only several hours before the physical findings, whereas the pathological process must consume more than 24 hours before the physical findings become apparent. He states that we cannot draw conclusions as to the course of the disease by determining the virulence on mice.

Graffagnini injected 1 gm. of sputum and 1 gm. of normal salt solution subcutaneously into mice. The injections were made every day during the height of the disease, and every second or third day after the fall of temperature. Forty cases were studied. In all the maximum virulence occurred during the first four days, after which there was a tendency of the virulence curve to drop, except in fatal cases, in which mice died in seven to eight hours. Whether the temperature fell by crisis or by lysis, the virulence of the sputum always followed a uniform course, diminishing gradually, but never decreasing with a suddenness comparable to the critical fall of the temperature. This led him to conclude that crisis is not due to a diminution of the virulence of the pneumococci, but to the slowly accumulating powers of defense in the body. The following experiments show the results obtained:

Experiment 1. 8th day crisis, mouse died in 12 hours.
 12th " " " " 24 "
 16th " " " survived.

Experiment 2. 6th day crisis, mouse died in 15 hours.
 8th " " " " 20 "
 9th " " " " "
 10th " " " " 24 "
 12th " " " " 36 "
 15th " " " survived.

This review has shown the great diversity of results obtained by the different observers, which diversity is probably due to difference in methods employed. With two exceptions, the pneumococcus was isolated by inoculating animals. Many based their results simply on inoculation of sputum. Arbitrary doses were chosen by some, while others used variable doses. If a large enough dose is injected, all cultures may kill, and hence it is

necessary not only to establish an arbitrary dose, but to determine a minimal lethal dose.

Statistics of comparative virulence of strains isolated by animal inoculation and subsequently tested on the same species by inoculating a pure culture are misleading, for we know that animal inoculation enhances the virulence, but not necessarily to the same degree. It has been noted that in some strains a few passages will raise the pneumococcus to its maximum virulence, while in others it requires numerous passages, and in still others the virulence remains unchanged.

There are numerous objections to obtaining the virulence curve of the pneumococcus during the course of pneumonia by injecting into mice, at stated intervals, a definite weight of sputum. Mixed cultures are used. Toxic or aggressin-like substances are introduced. The number of bacteria vary in different specimens and different portions of sputum. This is especially true after crisis, when the pneumococci disappear from the sputum so rapidly that it is difficult to obtain a strain, whereas before crisis, the blood agar plates show almost a pure culture. This alone would account for the rapid fall of virulence after crisis as determined by this method. It is also difficult to follow the virulence after crisis because of the absence of sputum.

METHOD.

In this research the plate method has been employed in isolating the pneumococcus for the reasons already given. The argument usually brought forward against this method is that the pneumococcus rapidly loses its virulence on artificial media. To meet this objection the following authorities are cited.

Foa retained the virulence of the pneumococcus for 60 days in blood from a rabbit after inoculating the blood 24 hours and keeping it in a cool, dark place in a sealed glass tube.

Bunzl and Federn found that the pneumococcus grew luxuriantly in ascitic fluid and kept its virulence for three months.

E. Fraenkel and Reiche succeeded in keeping pneumococci virulent and viable for months by using agar, streaked with blood.

Spolverini retained the virulence of pneumococci in sputum from 55 to 140 days.

Eyre and Washbourn retained the original virulence of three cultures on blood agar 15, 25, and 63 days respectively.

Rosenow found that by using blood agar slants he could retain a moderate degree of virulence as long as 250 days. In two instances, a high grade of virulence was retained as long as 149 and 163 days respectively.

Carapelle and Gueli found that by growing the pneumococcus on blood serum, they could not only retain the virulence, but could gradually increase it.

My results agree with these findings. Three strains were kept on blood agar slants at 10° C. for 100, 109, and 111 days respectively without loss of the original virulence.

ISOLATION AND IDENTIFICATION.

The sputum was collected in sterile Petri dishes and plated immediately as follows: Selected portions of this sputum were washed in three to six successive solutions of sterile broth and macerated in a tube of broth. From this suspension two to four loops respectively were placed on two fresh blood agar plates and spread evenly over the surface by means of a sterile bent glass rod. To facilitate an even distribution of the bacteria, a few drops of broth were added to each plate. When expectoration had ceased, the culture was obtained by means of a bent swab which was introduced into the lower pharynx without touching any part of the mouth. This swab was then agitated in a tube of broth, and from this suspension, two plates were prepared. For identification, I relied on the characteristic green colonies on blood agar plates as described by Schottmüller and Rosenow, the ring-shaped colonies described by Buerger, the growth in milk, the fermentation and coagulation of inulin-serum water, the morphology, and the capsule stain.

DOSAGE.

The injection of a definite weight of sputum is open to the objections stated above. The usual method is to take a certain portion of a growth from solid media, such as a loopful; a fraction of a 24-hour growth on an agar slant such as 1/10, 1/5, 1/2, etc.; or

if liquid media is employed, a certain number of c.c. or fractions of a c.c. of a 24-hour growth. The objection to these two methods is that the pneumococcus is very susceptible to the slightest difference in reaction, or quality of the medium. It was noted many times in this investigation that even slight elevations of the temperature above 37° C. prevented growth. Kruse and Pansini, Eyre, Leathem, and Washbourn, and others found that the most virulent strains were those which were the most delicate and sensitive on artificial media, and that the less virulent ones were much less delicate, and could flourish under conditions in which the virulent strains were unable to grow. There is also a marked difference in the rate of growth of different strains.

The nephelometers of McFarland, and of Wells and Richards, were considered. One objection to nephelometers is that the error varies with the density of the solution and is adaptable only to low dilutions. On account of the small quantity of fluid to be injected, the emulsions used in this research had to be relatively heavy. Another objection is that it is difficult not to rub off some of the medium or to get away from the coloring of the blood agar, all of which vitiate the result.

There are two methods of direct enumeration, that of Winslow and Willcomb, and that of Wright. Winslow and Willcomb give a very good idea of the comparative value of counting the number of bacteria by the plate method, and by the direct enumeration on coverslip. The plate method was not available for this research for obvious reasons.

In this work Wright's method has been chosen because it is the most accurate known to the writer. One thousand red cells were counted, and a blood count was made each time from the same drop of blood from which the slide was prepared.

The pneumococcus is very readily emulsified, so much so, that if a strain was obtained which showed any tendency to flake or clump, grave doubts were entertained as to its identity. With carefully prepared slides the error is therefore slight. Below are the actual figures of the first four counts of two observers, each counting 500 cells.

Red Cells	Bacteria
500	89
500	83
500	99
500	85
500	45
500	55
500	49
500	52

As only 24-hour growths were used nearly all of the organisms were alive.

After determining the number of bacteria per c.mm., the emulsion was diluted with normal saline solution so that 1/20 c.c. represented 20,000,000 bacteria. This was done by drawing the necessary quantity of the emulsion into a 1 c.c. Sub. Q. tuberculin syringe, graduated into 100 parts as described below, and then rinsing out the syringe with the quantity of normal saline needed to bring the emulsion to the standard required.

Five mice were injected with 20, 40, 80, 160, and 320 million bacteria respectively. By this means, the variations in the individual susceptibility of the animals was controlled. The lowest dose selected was one which was not fatal, or fatal only after many days, usually as a result of toxemia. By considering the time of death, a composite curve of virulence could be constructed.

AMOUNT OF FLUID INJECTED.

The amount of fluid injected should be small. If one liter of normal saline is introduced subcutaneously into a man weighing 150 pounds, 1/57 of his body weight has been injected; while 1 c.c., into a mouse weighing 20 gms., is equal to 1/20 of its body weight. The quantity of fluid injected should certainly not exceed this. Large amounts of saline also have an aggressin-like action.

METHOD OF INOCULATING PRECISE AMOUNTS.

A 1 c.c. safety Sub. Q. tuberculin syringe graduated into 100 parts was used. Syringes were selected with a caliber of about four mm. This made the calibrated portion of a 1 c.c. syringe

about 80 mm. long. The syringe was divided into 20 main divisions, making each division, or $1/20$ c.c. column, four mm. long, and hence not difficult to read.

Rosenau has shown that the loss in the mixing graduate is, on an average, about .0192 per cent. This loss would hold only if the dose for each mouse were in a separate container. The emulsion was made in a 5 c.c. test tube. After being standardized to 20,000,000 bacteria in $1/20$ c.c., the syringe was loaded from this emulsion for all injections. Four mice could be injected from one loading, i.e., $1/20$ c.c., $2/20$ c.c., $4/20$ c.c., and $8/20$ c.c. respectively. The fifth mouse received $16/20$ c.c., and reloading of the syringe from the same emulsion was necessary.

The loss in the syringe is from leaks at joints and at packing, from contact with glass and with surface of packing. By first drawing some air into the syringe, and then loading it with the emulsion, the leak at packing of piston, and also the loss by contact with packing was avoided. By using this *air cushion*, a meniscus was formed at junction of fluid and air, making the reading absolutely accurate. By simply twisting or rotating the piston, no difficulty was experienced in stopping at any one point. The usual method has been to use a rubber bulb in place of the piston, but a bulb is difficult to control, and by its use air is often introduced into the animal. The loss at needle end of syringe is obviated by having the thread molded into the glass, so that there are no connections to leak. The loss by adherence to sides of glass must be constant with each series, and would vary in the different series only if the glass were not clean.

MANNER OF CONSTRUCTING CHARTS.

The virulence curve is placed above the temperature curve, using the temperature line of 108 as the axis of abscissae. The points on the ordinates below, represent the number of hours in which the animal died from a given dose. Every degree of temperature represents 24 hours. Each point on the abscissae represents the hour of the day at which the sputum was collected.

HISTORIES AND PROTOCOLS.

In the first experiments, complete protocols are given showing the time of isolation, number of transfers made before inoculation, and the method of the standardization of the emulsion. All cultures fermented and coagulated inulin-serum water unless otherwise stated. Daily transfers were made on fresh blood agar tubes, and 24-hour growths were used for inoculation.

Case 1.—Acute lobar pneumonia. Left lower lobe. Child, aet. 11. History: Onset March 23 with otitis media, patient was deaf, and the drum head was inflamed and bulging. March 24, patient complained of a severe pain in left side and epigastric region. No physical signs were obtained by percussion or auscultation. March 25, sputum was prune juice in color, the leukocytes were 40,000, and physical signs became evident in the left lower lobe. March 26, about 8 p.m. patient had a typical crisis, and temperature dropped to normal in four hours, with profuse perspiration. March 27, there was a slight rise without any complications, and patient made an uneventful recovery.

EXPERIMENT 1.

March 25. 8 A.M. The sputum which was tenacious and prune juice in color, was collected into a sterile test tube and immediately plated on two blood agar plates. March 26. Two typically green colonies were isolated from plates and transplanted to blood agar tubes.

March 27. From the blood agar tubes, transfers were made to blood agar, milk, and inulin-serum water.

March 28. Milk was typically acid, the inulin fermented, and the serum water coagulated. From the 24-hour growth of the blood agar tubes, a thick emulsion was made in 1 c.c. normal salt solution. By means of a capillary pipette, equal parts of emulsion, blood, and saline were thoroughly mixed on a slide, and from this smears were made on two slides. These were stained with Hasting's stain. There were 5,336,000 red cells per c.mm. in the drop of blood used. In an equal number of fields on slide, there were 1,046 red cells and 58 bacteria. Hence in 1 c.mm. of red cells there were 295,877 bacteria. In 50 c.mm. of red cells (one main division of tuberculin syringe, or 1/20 c.c.) there were 14,793,850 bacteria. To standardize the emulsion so that one main division of tuberculin syringe, or 1/20 c.c. is equal to 10,000,000 bacteria, take $\frac{10,000,000}{14,793,850}$ or 0.675 c.c.

emulsion, or

67.5 marks of a tuberculin syringe graduated into 100 parts of emulsion.

32.5 " " " " " " " " 100 " " salt solution.

1 main division of syringe, or 1/20 c.c. = 10,000,000 bacteria.

2 " " " " " " " " 2/20 " = 20,000,000 "

4 " " " " " " " " 4/20 " = 40,000,000 "

8 " " " " " " " " 8/20 " = 80,000,000 "

March 28. Each of the above doses was injected into a mouse weighing 18 gms.

"	2	"	20,000,000	"	"	"	22	"
"	3	"	40,000,000	"	"	"	48	hours.
"	4	"	80,000,000	"	"	"	28	"

Mouse 1 died from hemorrhage into the pleural cavity. No organisms were recovered. Mice 3 and 4 showed numerous capsulated diplococci in smears and pure cultures were recovered from the heart's blood.

March 27. Twelve hours after crisis, the expectoration from lungs was collected into a sterile test tube, and plated immediately on blood agar.

March 28. Two typically green flat colonies were transferred to blood agar.

March 29. From the blood agar tubes, transfers were made to blood agar, inulin-serum water, and milk.

March 30. The inulin was fermented, the serum water coagulated, and the milk acid. Transfers were made from the blood agar to several blood agar tubes.

March 31. An emulsion was made and the slides prepared as in the preceding experiment.

Hence in 1 c.mm. of emulsion there were 1,628,524 bacteria.
 " 1/20 C.C. " " " " 81,426,200 "

I6 " " " " " I6/20 " = 320,000,000 "

“ 5 “ 320,000,000 “ “ “ 48 “

Mouse 1 had a large, fresh, blood clot in each pleural cavity. Smears showed no organisms. Cultures on blood agar and milk remained sterile.

Mouse 2 showed numerous capsulated diplococci in blood from heart.

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Mouse 3 had a large free blood clot in right pleural cavity. Smears from heart blood showed numerous capsulated organisms.

Mouse 5 showed numerous capsulated diplococci in blood from heart.

EXPERIMENT 3.

March 29. Two days after crisis the sputum was plated on blood agar.

April 1. An emulsion was prepared and standardized as in the two preceding experiments, and injected into five mice weighing 20 to 22 gms.

Mouse 1 receiving 20,000,000 bacteria died in 10 days.

" 2 " 40,000,000 " " " 28 hours.

" 3 " 80,000,000 " " " 5½ days.

" 4 " 160,000,000 " " " 6 hours.

" 5 " 320,000,000 " " " 6 "

Autopsy records:

Mouse 1. A large blood clot was found in each pleural cavity. No organisms were seen in smears, and the transfers to blood agar remained sterile.

Mouse 2. No organisms were found in smears made from the blood.

Mouse 3. Transfers of blood from heart to milk and blood agar gave pure cultures in both.

Mice 4 and 5. Numerous capsulated organisms were found in smears from blood.

EXPERIMENT 4.

March 31. Four days after crisis the sputum was plated on blood agar.

April 3. An emulsion was prepared and injected into five mice, each weighing 15 gms.

Mouse 1 receiving 20,000,000 bacteria was alive 2 months later.

" 2 " 40,000,000 " died in 8 days.

" 3 " 80,000,000 " " " 6½ "

" 4 " 160,000,000 " was killed by accident.

" 5 " 320,000,000 " died in 12 days.

Autopsy records:

Mouse 2. A blood clot was found in the pericardial sac and in each pleural cavity.

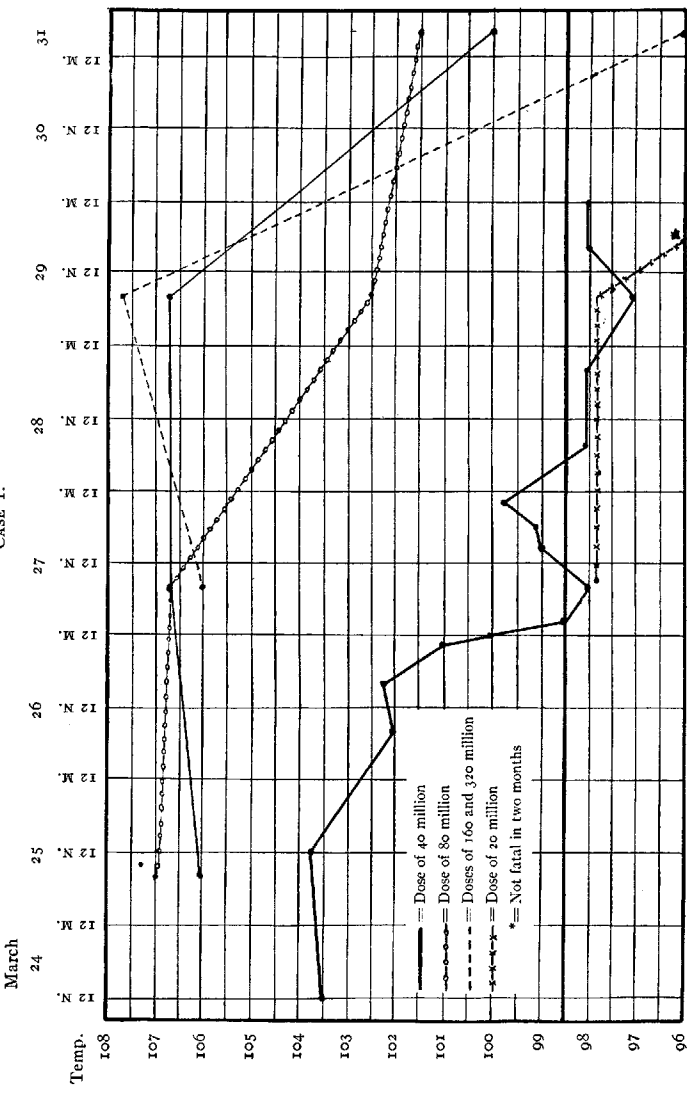
Pure cultures were recovered from the heart's blood and from the blood clots.

Mouse 5. A pure culture was recovered from the heart.

Chart 1 is a graphic representation of the temperature and virulence curves. While there was a typical crisis on March 26, the virulence of the organisms isolated on March 27 and 29 remained unchanged. The organisms isolated on March 31 were decidedly less virulent, as shown by experiment 4 and representation on chart.

Case 2.—J. S., aet. 44, carpenter. Acute lobar pneumonia, right lower lobe. Present illness began March 21 with a chill lasting two hours. The next day the patient had another chill, and developed a cough with pain in the right side. The sputum was streaked with blood. There were no complications, and the disease terminated by lysis with a slight elevation until April 11, due to delayed resolution.

CHART I.
CASE I.



* The dose of 20 million was continued until March 31; not fatal in 2 months.

EXPERIMENT 1.

March 28. The sputum was plated on blood agar.

March 31. Five mice were inoculated each weighing 20 gms.

Mouse 1	receiving	20,000,000	bacteria	died in	6 days.
" 2	"	40,000,000	"	"	" 1 day.
" 3	"	80,000,000	"	"	" 2 days.
" 4	"	160,000,000	"	"	" 1 day.
" 5	"	320,000,000	"	"	" 2 days.

Autopsy records: Numerous capsulated diplococci were found in the smears, and pure cultures were recovered on blood agar.

EXPERIMENT 2.

April 6. The sputum was plated on blood agar.

April 9. The standardized emulsion was injected into five mice.

Mouse 1	weighing	19 gms.,	received	20,000,000	bacteria	and died in	15 days.
" 2	"	20	"	40,000,000	"	"	" 12 hours.
" 3	"	18	"	80,000,000	"	"	" 12 "
" 4	"	23	"	160,000,000	"	"	" 5 days.
" 5	"	23	"	320,000,000	"	"	" 12 hours.

Autopsy records:

Mouse 1. A large blood clot was found in the pleural cavity, from which a pure culture of diplococci was recovered on blood agar.

Mice 2, 3, and 5 had free blood clots in the pleural cavities, and also hemorrhage into the pericardium. No organisms were recovered on transfers to blood agar.

Mouse 4. Capsulated diplococci were found in the smears from the blood, and a pure culture was recovered on blood agar.

EXPERIMENT 3.

April 10. The sputum was plated on blood agar.

April 16. An emulsion was injected into five mice each weighing 15 gms.

Mouse 1	receiving	20,000,000	bacteria	died in	48 hours.
" 2	"	40,000,000	"	"	" 48 "
" 3	"	80,000,000	"	"	" 48 "
" 4	"	160,000,000	"	"	" 48 "
" 5	"	320,000,000	"	"	" 48 "

These mice were accidentally exposed to a cold wind and rain from an open window, which may account for the uniform time of death.

Autopsy records: Numerous capsulated diplococci were found in smears, and pure cultures were recovered on blood agar.

EXPERIMENT 4.

April 17. The sputum was plated on blood agar.

April 22. Five mice, each weighing 18 to 20 gms., were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in	10 days.
" 2	"	40,000,000	"	"	" 10 "
" 3	"	80,000,000	"	(not fatal)	
" 4	"	160,000,000	"	"	"
" 5	"	320,000,000	"	"	"

Autopsy records:

Mouse 1 had a large hemorrhage into the pleural cavity. The thorax and neck were placed in Zenker's fluid for a pathological study as to the origin of the hemorrhage. Mouse 2 had a hemorrhage into the pleural cavity. No organisms were found in smears, and transfers from blood clot and heart remained sterile.

Chart 2 represents the temperature and virulence curves. The virulence remained unchanged until the temperature became normal. The probable explanation is that as long as physical signs are present, and pneumococci are expectorated from the lungs, the organisms remain virulent. When the physical signs disappear only such organisms as normally inhabit the mouth are obtained, and these become relatively avirulent because of some unfavorable environment. It is important to remember that the composition of saliva and that of sputum expectorated during the pneumonic process are entirely different; that while the pneumococcus may be kept alive for weeks in sputum, it rapidly dies in saliva.¹

Case 3.—R. T., aet. 11. Acute lobar pneumonia, right upper lobe. Present illness began March 27 with a severe chill. Twelve hours later the patient became delirious, and on the following day, the sputum was blood tinged. The crisis occurred on the sixth day. There were no complications.

EXPERIMENT 1.

March 31. The sputum was plated on blood agar.

April 3. An emulsion was injected into five mice, each weighing 24 gms.

Mouse 1 receiving 20,000,000 bacteria died in 2 days.

" 2 " 40,000,000 " " 2½ "

" 3 " 80,000,000 " " 28 hours.

" 4 " 160,000,000 " " 3 days.

" 5 " 320,000,000 " " 36 hours.

Autopsy records: Numerous capsulated diplococci were found in smears from the blood.

EXPERIMENT 2.

April 2, 5:30 P.M. The blood-tinged sputum was plated on blood agar.

April 5. An emulsion was injected into five mice, each weighing 20 gms.

Mouse 1 receiving 20,000,000 bacteria remained alive.

" 2 " 40,000,000 " died in 24 hours.

" 3 " 80,000,000 " " 28 "

" 4 " 160,000,000 " " 24 "

" 5 " 320,000,000 " " 24 "

Autopsy records: Numerous capsulated diplococci were found in smears from the blood.

EXPERIMENT 3.

April 6. The sputum was plated on blood agar.

April 10. Five mice, each weighing 18 gms., were inoculated.

Mouse 1 receiving 20,000,000 bacteria died in 24 hours.

" 2 " 40,000,000 " " 24 "

" 3 " 80,000,000 " " 26 "

" 4 " 160,000,000 " " 24 "

" 5 " 320,000,000 " " 24 "

Autopsy records: Numerous capsulated diplococci were seen in the smears from blood.

¹ For experiments in support of this view, see p. 318.

EXPERIMENT 4.

April 12. As the patient had ceased to expectorate, a broth suspension was made from a pharyngeal swab and plated on blood agar.

April 15. An emulsion was injected into four mice, each weighing 18 gms.

Mouse 1 receiving 40,000,000 bacteria died in 2 days.

" 2 " 80,000,000 " " 3 "

" 3 " 160,000,000 " remained alive.

" 4 " 320,000,000 " died in 4½ days.

Chart 3 represents the temperature and virulence curves as constructed from the data of the experiments. While there was a most typical crisis on April 2, without subsequent rise of temperature, or complication, the virulence of the organisms obtained from the sputum, nine hours after crisis, and four days after crisis, was the same as before crisis. With the disappearance of physical signs the patient ceased to expectorate, consequently the culture had to be made from a pharyngeal swab. This culture was relatively less virulent.

The notes made in the history in regard to the physical signs were as follows:

April 7. The consolidated area at the right apex is clearing. There are still numerous rales.

April 9. There is still a slight impairment of note, only a few rales are heard.

April 10. Only a few rales are heard. These disappear after several deep breaths.

April 20. The lungs are entirely clear.

Case 4.—J. Y., aet. 43. Acute lobar pneumonia (right upper and lower). Delayed resolution.

Past history: General health excellent.

Present illness began March 22 with pain in the right side, especially on taking deep breaths. At the same time he developed a cough, and began to expectorate a mucopurulent and slightly blood-tinged sputum. Two days later, he had two severe shaking chills. The leukocytes varied from 25,000 to 43,000. The temperature remained slightly elevated after crisis due to delayed resolution.

EXPERIMENT 1.

March 29. A fibrinous cast, expectorated into a sterile test tube, was plated immediately on blood agar plates, and on the following day the plate showed a pure culture of green pneumococci colonies.

April 3. Five mice, each weighing 15 gms., were inoculated.

Mouse 1 receiving 10,000,000 bacteria died in 31 days.

" 2 " 20,000,000 " remained alive.

" 3 " 40,000,000 " died in 2 days.

" 4 " 80,000,000 " " 2 "

" 5 " 160,000,000 " " 36 "

Autopsy records:

Mice 3 and 4 showed capsulated diplococci in smears, and pure cultures were recovered from the blood.

Mouse 1 had a large blood clot in the pleural cavity. No organisms were recovered from the blood.

Mouse 5 had a very large liver, studded with white areas. Smears showed numerous bacilli, possibly an invasion after death. The pneumococcus was not recovered.

EXPERIMENT 2.

March 31. The sputum was plated at 6 P.M.

April 3. An emulsion was injected into five mice, each weighing 15 gms.

Mouse 1	receiving	10,000,000	bacteria	died in	5 days.
" 2	"	20,000,000	"	"	" 3 "
" 3	"	40,000,000	"	"	" 2 "
" 4	"	80,000,000	"	"	" 18 hours.
" 5	"	160,000,000	"	"	" 18 "

Autopsy records:

Mouse 1. A pure culture was recovered from the blood.

Mice 2 and 3. Capsulated diplococci were found in smears from the blood, and pure cultures were recovered.

Mice 4 and 5. Numerous capsulated diplococci were found in smears, and pure cultures were recovered from the blood.

EXPERIMENT 3.

April 4. The sputum was plated on blood agar.

April 8. An emulsion was injected into five mice, each weighing 15 gms.

Mouse 1	receiving	20,000,000	bacteria	remained	alive.
" 2	"	40,000,000	"	died in	24 hours.
" 3	"	80,000,000	"	"	" 24 "
" 4	"	160,000,000	"	"	" 24 "
" 5	"	320,000,000	"	"	" 24 "

Autopsy records: Numerous capsulated diplococci were found in the smears.

EXPERIMENT 4.

April 18. The sputum was plated on blood agar.

April 24. Five mice, each weighing 15 gms. were inoculated.

Mouse 1	receiving	20,000,000	bacteria	died in	2 days.
" 2	"	40,000,000	"	"	" 2 "
" 3	"	80,000,000	"	"	" 6 "
" 4	"	160,000,000	"	"	" 3 "
" 5	"	320,000,000	"	remained	alive.

Autopsy records:

Mice 1 and 2. Few organisms were seen in smears. A pure culture was recovered from the blood.

Mouse. 4. Numerous capsulated diplococci were seen in smears. A pure culture was recovered in the blood.

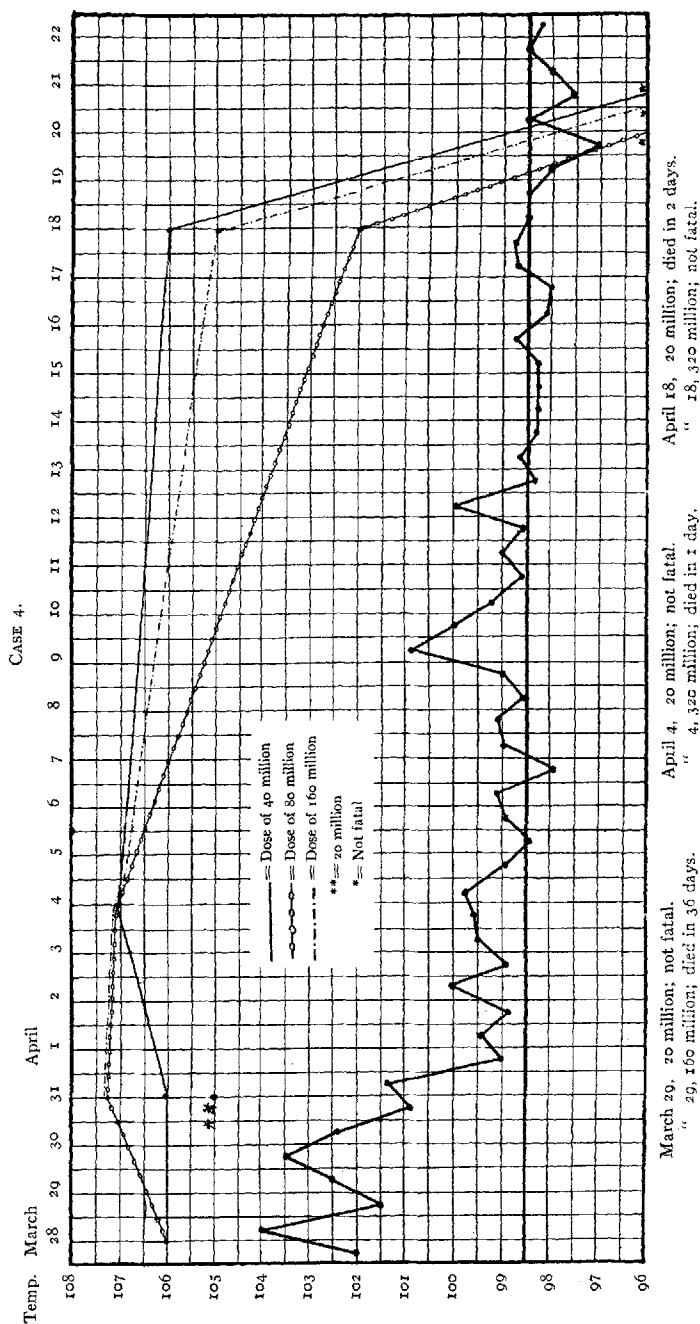
EXPERIMENT 5.

April 22. A broth suspension was made from a swab from the pharynx and plated immediately on blood agar. On the following day three green colonies were isolated. There was a typical growth in milk, but the inulin-serum water was not coagulated or fermented. The smears showed lanceolate diplococci which stained by Gram's method.

April 26. The growth on two blood agar slants was injected into a mouse without producing a fatal result.

Chart 4 shows the termination of the disease to be by lysis. The delayed resolution accounts for the rise of temperature until April 13. The virulence curve

CHART 4.



shows that the organisms isolated during lysis were as virulent as those obtained during the active process. This virulence did not decrease until resolution was complete, and the temperature remained normal.

Case 5.—R. B., aet. 12. Acute lobar pneumonia (lower left). Present illness began April 15, four days before admission, with headache, fever, and pain all over body. On the fourth day patient was brought in an ambulance to the hospital. She had a typical crisis on the eighth day.

EXPERIMENT 1.

April 19. The blood-tinged sputum was plated on blood agar. On the following day, the plates showed an almost pure culture of green colonies. The growth was rather profuse and mucoid, and the plates appeared as though a small amount of water had been allowed to flow over the surface. The cultures isolated were of a variety of the pneumococcus usually called *Streptococcus mucosus capsulatus*. All of the colonies fermented and coagulated inulin, and rapidly acidified milk with late coagulation.

April 21. Five mice, each weighing 16 gms., were inoculated.

Mouse 1 receiving 20,000,000 bacteria died in 2 days.

" 2 " 40,000,000 " " " 2 "

" 3 " 80,000,000 " " " 2 "

" 4 " 160,000,000 " " " 1 day.

" 5 " 320,000,000 " " " 6 hours.

Autopsy records: All of the mice showed numerous capsulated diplococci in smears.

EXPERIMENT 2.

April 21. 1 P.M. The sputum was plated. The resultant growth showed the same variety as that in experiment 1.

April 24. Five mice, each weighing 18 to 20 gms., were inoculated.

Mouse 1 receiving 20,000,000 bacteria died in 36 hours.

" 2 " 40,000,000 " " " 36 "

" 3 " 80,000,000 " " " 24 "

" 4 " 160,000,000 " " " 36 "

" 5 " 320,000,000 " " " 36 "

Autopsy records: Smears from the blood showed large numbers of large capsulated diplococci.

EXPERIMENT 3.

April 22. The sputum was collected at 1 P.M. and plated on blood agar.

April 23. The plates showed a pure culture of green colonies of the variety *Streptococcus mucosus capsulatus*. Five mice, each weighing 15 gms., were inoculated.

Mouse 1 receiving 20,000,000 bacteria died in 36 hours.

" 2 " 40,000,000 " " " 36 "

" 3 " 80,000,000 remained alive.

" 4 " 160,000,000 died in 36 hours.

" 5 " 320,000,000 " " " 24 "

Autopsy records: All of the mice showed numerous capsulated diplococci in smears from the blood.

EXPERIMENT 4.

April 26. No expectoration was obtainable at the hour when it was wanted, so a broth suspension was made from a pharyngeal swab and plated on blood agar. On the following day, three colonies were isolated, all rapidly acidified milk, with partial coagulation in four days. The inulin was not fermented or coagulated. Smears showed typical lance shaped diplococci which stained by Gram's method.

April 30. Five mice, each weighing 15 gms., were inoculated.

Mouse 1 receiving 20,000,000 bacteria died in 36 hours.

"	2	"	40,000,000	"	"	"	36	"
"	3	"	80,000,000	"	"	"	18	"
"	4	"	160,000,000	"	"	"	18	"
"	5	"	320,000,000	"	"	"	18	"

Autopsy records: Capsulated diplococci were seen in smears. Mouse 5 had a large hemorrhage into the pleural cavity.

EXPERIMENT 5.

May 1. A broth suspension was made from a pharyngeal swab, and plated on blood agar.

May 2. The plates showed a practically pure culture of green colonies. Three colonies were isolated, two of which fermented and coagulated inulin-serum water. The milk was rapidly acidified and partially coagulated within 24 hours. The smears showed lance shaped diplococci which stained by Gram's method.

May 4. Five mice, each weighing 15 gms., were inoculated.

Mouse 1 receiving 20,000,000 bacteria died in 24 hours.

"	2	"	40,000,000	"	remained alive.
"	3	"	80,000,000	"	remained alive.
"	4	"	160,000,000	"	died in 24 hours.
"	5	"	320,000,000	"	" " 36 "

Autopsy records: Capsulated diplococci were found in smears from the blood.

Chart 5 shows that while a typical crisis occurred on April 20, the strains isolated one, two, and six days after crisis were as virulent as those isolated on the day before crisis. It also shows that there is no difference in virulence between the cultures obtained by pharyngeal swabs (experiment 4) and those obtained from the sputum expectorated (experiments 2 and 3). When the lung signs had cleared, the strain isolated from the pharynx was less virulent, two of the five mice inoculated surviving.

Case 6.—P. H., aet. 35. Acute lobar pneumonia (right upper, middle, and lower lobes). Present illness began March 31, with pain in back and right side. During the night a cough developed accompanied by a very severe headache. From that time the patient had fever, pain, and dyspnea. The sputum became blood tinged on April 5, two days after admission.

EXPERIMENT 1.

April 6. The blood-tinged sputum was plated on blood agar.

April 7. The plates were covered with colonies, majority of which were green. Several of these were isolated, and on the following day transferred to milk, inulin, and blood agar. The milk was rapidly acidified, but the inulin was only

partially fermented or coagulated. The organisms appeared as lance shaped pairs, but there was a marked tendency to form short chains. They stained by Gram's stain.

April 10. Five mice, each weighing 18 gms., were inoculated.

Mouse 1 receiving 20,000,000 bacteria died in 2 days.

" 2	"	40,000,000	"	"	" 2	"
" 3	"	80,000,000	"	"	" 5	"
" 4	"	160,000,000	"	"	" 3	"
" 5	"	320,000,000	"	"	" 6	"

Autopsy records:

Mice 1, 2, and 3 showed numerous capsulated diplococci in smears from the blood.

Mouse 2 had a blood clot in the pleural cavity.

Mouse 4 did not show any organisms in smears from the blood.

Mouse 5 had a blood clot in the pleural cavity. No organisms were found in smears.

EXPERIMENT 2.

April 9, 5 P.M. The sputum was plated on blood agar. On the following day four green colonies were isolated. These rapidly acidified milk, but did not ferment or coagulate inulin-serum water. The organisms appeared as lance shaped diplococci with the same tendency to chain formation as in experiment 1. They stained by Gram's method.

April 13. Five mice, each weighing 18 gms., were inoculated.

Mouse 1 receiving 20,000,000 bacteria died in 4 days.

" 2	"	40,000,000	"	"	" 8	"
" 3	"	80,000,000	"	"	" 3	"
" 4	"	160,000,000	"	"	" 4	"
" 5	"	320,000,000	"	"	" 6	"

Autopsy records:

Mice 1 and 4 were lost by accident after death.

Mice 2 and 5 had a large blood clot in the pleural cavity. No organisms were recovered by transfers to blood agar.

Mouse 3 did not show any organisms in smears from the blood.

EXPERIMENT 3.

April 11. The sputum was collected at 10 A.M. and plated on blood agar. On the following day, three typically green colonies were isolated, and transferred to milk, inulin, and blood agar. The milk was rapidly acidified, but the inulin-serum water was not coagulated or fermented. The organisms appeared as lance shaped diplococci, many of which formed short chains. They stained by Gram's method.

April 14. Five mice, each weighing 20 gms., were inoculated.

Mouse 1 receiving 20,000,000 bacteria died in 6 days.

" 2	"	40,000,000	"	remained alive (May 18).
" 3	"	80,000,000	"	died in 7 days.
" 4	"	160,000,000	"	" " 4 "
" 5	"	320,000,000	"	" " 4 "

Autopsy records:

Mouse 1 had a hemorrhage into the pleural sacs. No growths resulted from transfer of blood to blood agar.

Mouse 3. No organisms were recovered from the heart.

Mice 4 and 5 were lost by accident after death.

Chart 6 represents the temperature and virulence curves. Although a typical crisis occurred on April 9, the organisms isolated from the sputum during the crisis, and two days after the crisis, were as virulent as those before the crisis. No later cultures were taken in this case. The strains isolated from this case are unusual in that they did not ferment or coagulate inulin-serum water and showed a decided tendency to chain formation on blood agar. The chart also shows a very unusual low grade of virulence. With the exception of capsule formation in the animal (experiment 1) these cultures resemble the type of *Streptococcus viridans* described by Schottmüller.

Case 7.—G. S., aet. 17. Acute lobar pneumonia (right upper and lower lobes, left upper and lower lobes). Present illness began March 25, with profuse perspiration. The next day, a severe pain developed in the left side and abdomen. Three days after onset, the expectoration became blood tinged. The patient had no chills or convulsions. The leukocytes reached 72,000 on April 4. Crisis occurred on April 6.

EXPERIMENT 1.

April 1. The sputum was plated.

April 5. Five mice, each weighing 20 to 22 gms., were inoculated. (Mouse 5 weighed 27 gms.)

Mouse 1 receiving	10,000,000	bacteria died in 48 hours.
" 2 "	20,000,000	" remained alive.
" 3 "	40,000,000	" died in 16 days.
" 4 "	80,000,000	" " " 3 "
" 5 "	160,000,000	" " " 9 "

Autopsy records:

Mouse 1. Myriads of capsulated diplococci were found in smears from the blood. A pure culture was recovered from the heart on blood agar.

Mouse 3 had been suffering from a paralysis for days. No hemorrhage was noted, and no organisms were recovered.

Mouse 4. Few organisms were seen in the smears. A pure culture was recovered from the blood.

EXPERIMENT 2.

April 4. The sputum was plated.

April 8. Four mice, each weighing 20 to 22 gms., were inoculated.

Mouse 1 receiving	20,000,000	bacteria died in 36 hours.
" 2 "	40,000,000	" " " 36 "
" 3 "	80,000,000	" " " 36 "
" 4 "	160,000,000	" " " 36 "

Autopsy records:

Mouse 1 had a large hemorrhage into the pericardium.

Mouse 2 had a large free blood clot in the pleural cavity.

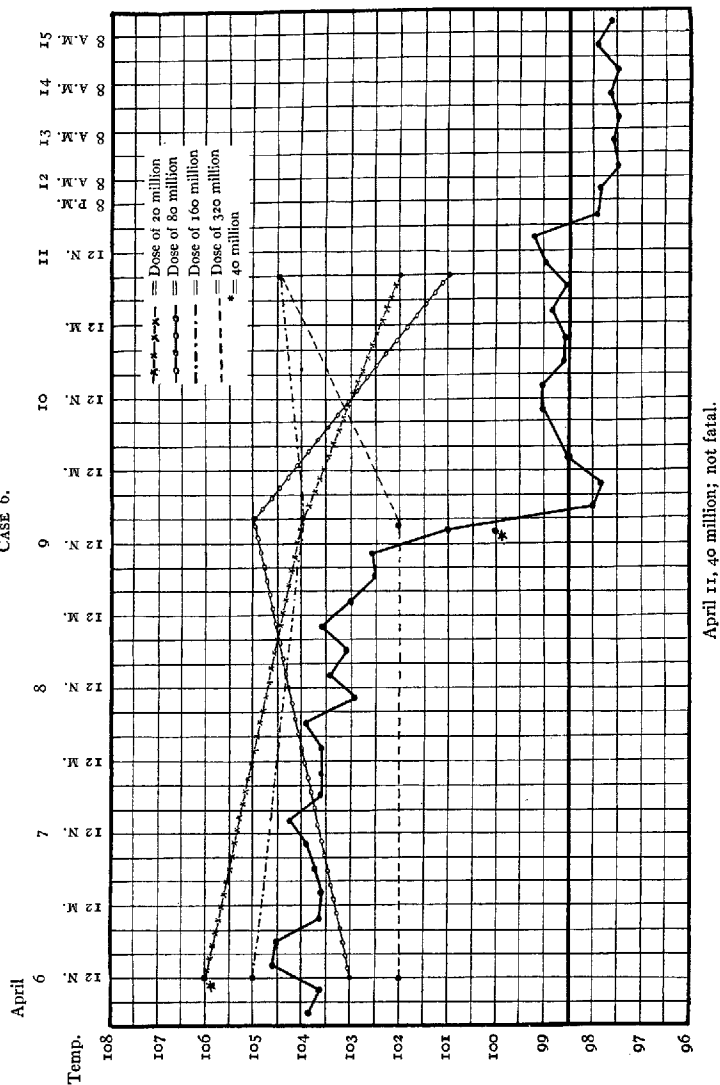
Pure cultures of capsulated diplococci were recovered from the blood in all of the mice.

EXPERIMENT 3.

April 7. The sputum was collected 24 hours after crisis, and plated on blood agar.

April 9. Five mice, each weighing 25 to 28 gms., were inoculated.

CHART 6.
CASE 6.



Mouse 1 receiving	20,000,000	bacteria died in 12 hours.
" 2 "	40,000,000	" " " 12 "
" 3 "	80,000,000	" " " 36 "
" 4 "	160,000,000	" " " 5½ days.
" 5 "	320,000,000	" " " 36 hours.

Autopsy records:

Mouse 1 had a hemorrhage into the pericardium.

Mice 1, 2, and 5. Capsulated diplococci were found in smears, and a pure culture was recovered on blood agar.

Mouse 3 had a large blood clot in the left pleural cavity, also a hemorrhage into the pericardium. Smears showed numerous capsulated diplococci.

Mouse 4 had a hemorrhage into the pericardium. A pure culture was recovered on blood agar. The sputum was plated on the 15th and 18th, but the cultures isolated were discarded because the strains were not typical.

EXPERIMENT 4.

April 19. The sputum was plated on blood agar, and on the following day, six green colonies were isolated.

April 21. Transfers were made to milk and inulin. Three of the strains gave a typical growth in milk, one strain constantly fermented inulin to a slight degree, but did not coagulate the serum.

April 24. A mouse was inoculated subcutaneously with the growth from two blood agar slants.

April 26. Mouse remained alive. The growth from two tubes was again injected.

May 4. The mouse died from a hemorrhage. A large blood clot was found in the pleural cavity. No organisms were recovered in smears or on transfers to blood agar.

(A young mouse, four weeks old, was inoculated with the growth obtained from two blood agar tubes. Death occurred in 18 hours, and myriads of definitely capsulated diplococci were seen in the smears.)

These experiments show that the virulence of the pneumococci isolated on the day after crisis was practically the same as before crisis. The virulence of the organisms isolated 13 days after crisis was of a very low grade.

Case 8.—L. C., aet. 29. Acute lobar pneumonia (left side.) Present illness began with a severe chill on April 18, followed by a sharp pain in the left side, and a marked shortness of breath. At this time he began to cough, expectorating a bloody sputum. The temperature varied from 102° to 105°. Lysis began on April 27, and the temperature was practically normal after May 2. There was a total suppression of the chlorides until April 29, with a marked rise after this date.

May 10. A broth suspension was made from a pharyngeal swab and plated on blood agar. On the following day six typical green colonies were isolated, all of which fermented and coagulated inulin-serum water, and acidified and coagulated milk. Smears showed a pure culture of lance shaped diplococci which stained by Gram's method.

April 15. Five mice, each weighing 15 to 17 gms., were inoculated.

Mouse 1 receiving	20,000,000	bacteria remained alive.
" 2 "	40,000,000	" " "
" 3 "	80,000,000	" died in 3 days.
" 4 "	160,000,000	" " " 1 day.
" 5 "	320,000,000	" remained alive.

Autopsy records:

Mouse 4 showed a large free blood clot in left pleural cavity. Smears from blood showed few organisms, which were capsulated. Blood agar cultures from the blood clot and heart gave pure cultures of pneumococci.

Mouse 3 had a large free blood clot in the right pleural cavity, also in right pericardium. The origin of the hemorrhage seemed to be from the mediastinum. Smears from the clot and heart did not show any organisms. Transfers to blood agar failed to produce any growth.

These strains, isolated from the throat after the physical signs had disappeared, had a relatively low grade of virulence.

Case 9.—M. S., aet. 15. Acute lobar pneumonia (left side). Present illness began April 11 with a chill, followed by fever, nausea, vomiting, headache, and a sharp pain in the left side. Three days after onset he started to cough up a bloody sputum. Crisis occurred on the day of admission, April 17. There were slight elevations of temperature to 99.5° and 100° until May 9, after which his temperature remained normal.

May 11. A broth suspension was made from a pharyngeal swab, and plated on blood agar. On the following day seven colonies were transferred to blood agar. Only one of these strains fermented inulin and coagulated serum water. Smears from this culture showed typical lance shaped diplococci which stained by Gram's method.

May 15. An emulsion was made from a 24-hour growth on blood agar, and injected into five mice each weighing 16 gms.

Mouse 1 receiving 20,000,000 bacteria remained alive.

"	2	"	40,000,000	"	"	"
"	3	"	80,000,000	"	died in 24 hours.	
"	4	"	160,000,000	"	"	" 48 "
"	5	"	320,000,000	"	remained alive.	

Autopsy records: Neither mouse showed any organisms in smears from heart, and no growth resulted from transfers to blood agar.

This experiment shows that a strain, isolated from the throat after the pneumonic process had cleared, had a relatively low grade of virulence.

On May 9, the hospital note on the condition of the lungs was as follows: "Lungs are clear throughout, except at left axilla, where the breath sounds are a little suppressed."

Case 10.—D. H., aet. 25. Acute arthritis of hip (infectious), chronic nephritis, hematuria. X-ray showed slight destruction of cartilage of joints. Temperature 99° to 101°.

May 10. A broth suspension was made from a pharyngeal swab and plated on blood agar. On the following day five typical green colonies were isolated, all of which fermented and coagulated inulin-serum water, and gave a typical growth in milk. Smears showed pure culture of lance shaped diplococci which stained by Gram's method.

May 14. Five mice were inoculated.

Mouse 1 (weight 17 gms.) receiving 20,000,000 bacteria remained alive.

"	2	"	17	"	"	40,000,000	"	"	"
"	3	"	18	"	"	80,000,000	"	died in 3 days.	
"	4	"	20	"	"	160,000,000	"	remained alive.	
"	5	"	21	"	"	320,000,000	"	died in 3 days.	

Autopsy records:

Mouse 5 had a large clot in the right pleural cavity.

Mouse 3 did not show any hemorrhage. The culture tubes to which transfers had been made were discarded by mistake.

This strain, obtained from a patient not ill with pneumonia, had the same relative virulence as the strains obtained from the throats of patients convalescing from pneumonia after the physical signs had disappeared.

Case II.—D. P., aet. 20. Admitted March 10, 1910; died March 30. Lobar pneumonia, right side; bronchopneumonia left side, chronic fibrinous pleurisy, cloudy swelling of viscera; acute endocarditis, aortic valve; ulceration into right ventricle; acute splenic tumor; purulent meningitis.

March 20. The sputum was plated on blood agar. On the following day three green colonies were isolated, all of which fermented and coagulated inulin-serum water, and gave a typical growth in milk.

March 25. An emulsion was made and injected into four mice.

Mouse 1 (weight 21 gms.) receiving 20,000,000 bacteria died in 4 days.

" 2 " 20 " " 25,000,000 " " " 3 "

" 3 " 19 " " 30,000,000 " " " 3 "

" 4 " 22 " " 40,000,000 " " " 3 "

March 26. An emulsion was made from an organism isolated from the blood on March 20 and injected into four mice.

Mouse 1 (weight 18 gms.) receiving 10,000,000 bacteria died in 5 days.

" 2 " 18 " " 20,000,000 " " " 3 "

" 3 " 18 " " 25,000,000 " " " 3 "

" 4 " 18 " " 30,000,000 " " " 4 "

Conclusions: From this case it would seem that there is no difference in the virulence of the organisms isolated the same day from the sputum and from the blood.

ACTION OF SALIVA ON THE PNEUMOCOCCUS.

Experiment 1.

The organism tested was obtained from a blood culture from a case of pneumonia. As much of a 24-hour growth as adhered to a bent platinum needle was emulsified in (1) a tube of broth (10 c.c.). Of this suspension, six loops were transferred to (2) a second tube of broth.

The saliva was filtered through a Berkfeld filter which had previously been sterilized in the autoclave. This filtrate was collected in a sterile tube, and cultures from it remained sterile.

A. 1 c.c. of (2) was added to 1 c.c. of filtered saliva.

B. 1 c.c. of (2) was added to 1 c.c. of salt solution.

Plated six loops of each A and B, on fresh blood agar plates; (a) immediately; (b) four hours later; (c) eight hours after (a); and allowed them to incubate 24 hours at 37° C.

(a)	Plated immediately	A = 11 colonies B = 20 "
(b)	" after four hours	A = 18 " B = too numerous to count
(c)	" " eight "	A = 8 colonies B = dry plate.

Experiment 2 (same technic).

(a)	Plated immediately	A = 38 colonies. B = 35 "
(b)	" after four hours	A = 53 " B = 80 "
(c)	" " nine "	A = 2 " B = 426 "
(d)	" " 21 "	A = 0 B = too numerous to be counted.

Sanarelli gives similar results for the staphylococcus with saliva filtered through a Chamberland filter.

From a staphylococcus culture as much as adhered to a needle was transferred to 10 c.c. of filtered saliva.

(a)	Plated immediately	225 colonies.
(b)	" after 12 hours	0 "
(c)	" " 24 "	0 "
(d)	" " two days	0 "
(e)	" " three days	0 "

He states that saliva is a good culture media for the pneumococcus, that the organisms multiply rapidly, but also lose their virulence rapidly. He does not give any counts for the pneumococcus. Grawitz and Steffen confirmed this progressive and rapid loss of virulence in saliva, but found that this virulence is rapidly regained in pneumonic sputum. Their results were not controlled by plate counts. The loss of virulence may have been due to death of many of the organisms.

HEMORRHAGE IN MICE INOCULATED WITH THE PNEUMOCOCCUS.

No attempt was made to follow and describe the pathological processes. An autopsy was made in each case, smears were made from the heart's blood, also capsule stains. Transfers were made to blood agar slants and milk, for the recovery of the organism.

A condition which was very unusual was the presence of a fresh large blood clot, usually in both pleural cavities, also a large clot

in the pericardial sac. The blood seemed to come from vessels at the base of the heart or the hilum of the lung.

A specimen was submitted to Dr. Thomas P. Sprunt, instructor in pathology, whose report is as follows:

"Specimen submitted for examination is the body of a white mouse with massive blood clots in the thorax. The thoracic organs were fixed *en masse* in Zenker's fluid, embedded in celloidin and serial sections studied. Every tenth section was stained with hematoxylin and eosin, others were treated with Weigert's elastic tissue stain.

"There are large masses of blood in all the serous cavities, in the mediastinal tissues, and smaller hemorrhages in the lungs. The cause of the hemorrhage is quite apparent in the remarkable lesions found in the larger blood vessels. One of the most conspicuous of these is found at the point where a large artery is branching from the aorta. In one section, the whole sectional area of this large artery is affected, in other sections only one-half or one-third of its circumference. The lesion appears to be of irregular shape approximately 0.5 mm. in diameter. In some sections the edge of the pathological zone is quite sharp; in others the wall of the vessel diminishes in thickness gradually, finally losing entirely its normal structure of smooth muscle and elastic tissue and becomes continuous with a narrow delicate pink-staining tissue. It shows a faint nucleus here and there. With Weigert's stain this area is very striking. The elastic tissue of the aorta stains intensely and stops abruptly at the edge of the lesion. In the thin delicate tissue already noted there appear only a very few fragmented elastic fibres. The lumen of the vessel and the surrounding tissues are filled with blood and show no difference in this respect. These hemorrhages extend along the vessels in the mediastinal tissues and along the pulmonary vessels into the lung. Similar lesions are found in the pulmonary arteries in the interlobar spaces. The walls of these vessels are sharply broken apart and the hole thus formed in the wall is occasionally filled by a thrombus composed largely of platelets. Except in these sharply circumscribed areas the walls of the vessels seem perfectly normal. The lesions described are mainly confined to

the arteries which are composed of elastic tissue and smooth muscle. The veins in this animal show only cardiac muscle in their walls for a considerable distance from the heart and are therefore easily distinguished. In one of the smaller pulmonary veins there is a little pouch-like protrusion from its wall which is covered by tissue similar to that in the large artery already mentioned. Within the lungs, blood is collected in greatest amounts around the large vessels, but there are also scattered hemorrhages in the lung alveoli. In association with these small hemorrhages, the alveolar walls are sometimes thickened and show many polymorphonuclear leukocytes. There are very few leukocytes within the alveoli. There is no apparent inflammatory process in the walls of the vessels. The lesion in the aorta and its large branch apparently consists of an atrophy of the tissues composing its walls. This atrophy is not so manifest in the smaller pulmonary arteries in which the rupture might possibly be explained by some sudden trauma." (Further studies on this condition are in progress.)

Out of 84 fatal cases, 24 show this large blood clot in one or all of the regions described above. The following tabulation gives the duration of life of the animals dying from hemorrhage after inoculation.

5 mice died in 1 day.	1 mouse died in 11 days.
4 " " " 2 days.	1 " " " 15 "
1 mouse died in 6 "	1 " " " 17 "
2 mice died in 8 "	1 " " " 19 "
2 " " " 10 "	5 mice " after 20 days.

The shortest period was one day, the longest 36 days. No organisms were recovered from the blood in the late cases. One case gave a positive culture as late as the sixth day, another as late as the eighth day.

EFFECT OF DOSE ON THE FREQUENCY OF HEMORRHAGE.

In seven, hemorrhage followed the injection of 20 million bacteria; in seven, the injection of 40 million; in four, the injection of 80 million; in one, the injection of 160 million; in three, the injection of 320 million. The reason for the hemorrhages occurring more frequently with the smaller doses is that the duration of life was longer as shown by the following:

Dose	Days before fatal hemorrhage occurred
20 million	2, 10, 10, 11, 15, 31, 36 days.
40 "	1, 1, 2, 8, 32 days.
80 "	1, 2, 2, 8 days.
160 "	6 days.
320 "	1, 2, 19 days.

CONCLUSIONS.

The following conclusions may be drawn from the experimental data of this investigation:

1. Crisis is not a result of any change in the virulence of the pneumococcus.
2. As long as pneumococci are expectorated from the lungs (for a short time in cases with crisis, for a longer period in cases with lysis) the organisms retain practically their original virulence.
3. Strains obtained from pharyngeal swabs during the pneumonic process have the same virulence as those obtained from the sputum.
4. Strains obtained from pharyngeal swabs after the lungs have resolved and expectoration has ceased, have a relatively low grade of virulence.
5. A strain obtained from the throat of a patient not ill with pneumonia, had the same relative virulence as strains obtained from the throats of convalescent patients in whom the physical signs had disappeared.
6. Loss of virulence of organisms obtained from the throats of patients who have recovered from pneumonia, seems to be due to an unfavorable action of the saliva.
7. From the results obtained in one case, there does not seem to be any difference in virulence of the organisms obtained from the blood, and those obtained from the sputum.

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