IMMUNITY FACTORS IN PNEUMOCOCCUS INFECTION IN THE DOG.

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PLATE 6.

(Received for publication, April 24, 1916.)

The varied native resistance that different animal species, or individuals of the same species, offer to bacterial infection has been the object of much investigation and also of speculation. This has naturally arisen from the *a priori* fact that a thorough knowledge of nature's methods of combating infections is an indispensable prerequisite of a successful treatment of infectious diseases. In our opinion, a study of the problem can be successfully approached by determining the fate of bacteria when inoculated into animals possessing widely different degrees of resistance.

Dogs have a comparatively high resistance to pneumococci, while rabbits succumb to an exceedingly small quantity of a virulent culture. When pneumococci are introduced into the circulation of rabbits, they soon begin to multiply and a fatal septicemia rapidly follows. Just what takes place in dogs under similar conditions has not been definitely determined and the mechanism of their high resistance is unknown. Hence, it was believed that a detailed investigation of the phenomena following intravenous injections of pneumococci in dogs might yield a fuller knowledge of infection and resistance in general.

Previous Observations.

Fraenkel states that dogs are quite refractory to intravenous inoculations of pneumococci.[1] Gamaléia claims that subcutaneous inoculations cause only local reactions while intrathoracic inoculations give rise to a lobar pneumonia similar

[1] Fraenkel, A., *Z. klin. Med.*, 1886, x, 401.

to that seen in man.[2] Monti showed that subdural injections of pneumococci produced typical meningitis in dogs.[3] It is claimed by Kruse and Pansini that subcutaneous inoculations of from 2 to 3 cc. of culture killed dogs within 2 to 5 days, while intravenous and intraperitoneal inoculations were without effect.[4] In a study of intrabronchial insufflations of pneumococci in dogs Lamar and Meltzer[5] observed that dogs receiving large quantities of culture developed persistent fever, did not eat, were prostrated, and died 2 to 4 days after the inoculation. These dogs gave positive blood cultures at autopsy but no detailed observations were made on the course of the septicemia. Wollstein and Meltzer[6] noted, moreover, that intrabronchial insufflations of virulent pneumococci gave rise to a bacteremia, while non-virulent cultures did not. Here, also, no quantitative observations were made.

From the literature it is readily seen that there is little definite knowledge concerning the fate of pneumococci injected into the circulation of dogs, and also that no systematic study has been made of the phenomena that the injections may incite.

The object of the present study was (1) to determine more definitely the relative resistance of dogs to pneumococcic infection, (2) to follow quantitatively the fate of the injected bacteria from the beginning to the end of the infection, and (3) to investigate the defensive mechanism and immunity responses on the part of the infected animals.

Technique.

Dogs were given intravenous inoculations of from 1 to 4 cc. per kilo of a bouillon culture of virulent[7] pneumococci. Cultures were made from the heart's blood as early as 1 minute after the injection and at short intervals for the first 6 hours and then at 24 hour periods. The blood was obtained from the heart and the cultures were made according to the method described in connection with making blood cultures from rabbits infected with pneumococci.[8] After the course

[2] Gamaléia, N., *Ann. Inst. Pasteur*, 1888, ii, 440.

[3] Monti, A., *Riforma med.*, 1889, v, 344, 350.

[4] Kruse, W., and Pansini, S., *Z. Hyg.*, 1892, xi, 279.

[5] Lamar, R. V., and Meltzer, S. J., *J. Exp. Med.*, 1912, xv, 133.

[6] Wollstein, M., and Meltzer, S. J., *J. Exp. Med.*, 1913, xvii, 353.

[7] The pneumococci were so virulent for rabbits that 0.00005 cc. killed within 24 hours. The virulence was maintained at this level by frequent passage in these animals.

[8] Bull, C. G., *J. Exp. Med.*, 1915, xxii, 457.

of the infections had been established in a general way, it was found that countable plates could be made with undiluted blood after the first hour, hence measured quantities of blood were put directly into the plates and melted agar was poured in before clotting occurred. At least three plates were made from each specimen of blood and the average figures recorded in terms of the number of colonies developing from 1 cc. of blood.

Course of the Septicemia.

Through the above procedure it was discovered that the curve of the blood cultures following intravenous inoculations of pneumococci in dogs is characteristic and presents several points of interest. The injected cocci leave the circulating blood rapidly. The mass of the bacteria disappears within the first 10 minutes, the cultures dropping from about 5,000,000 colonies per cc. 1 minute after the inoculation to 175,000 at 10 minutes after. From 3 to 6 hours after the inoculations the cultures show from 50 to 200 colonies per cc. of blood. At the end of 24 hours there is, as a rule, a slight increase over the 6 hour culture. At this point the septicemia may take one of two courses. The number of organisms may gradually decrease and the blood become sterile after 2 or 3 days, a true infection not arising. On the other hand, the septicemia may gradually increase for the next 24 or 48 hours and then rapidly ascend to a climax which is attained between the 4th and 5th days. At this point a downward direction is taken and the blood becomes sterile within from 1 to 4 days. This would probably be the end of the infection if it were not for the fact that a meningitis has invariably developed in the meantime. In a number of instances, however, the blood remained sterile in the presence of a severe, and, as a rule, fatal meningitis. Every fatal case had a severe pneumococcic meningitis. The following protocols illustrate the points mentioned.

Dog 1.—Weight 6 kilos. 6 cc. of a bouillon culture of pneumococci were injected into the ear vein and observations were made as given in Table I.

Autopsy.—The lungs are normal; the heart is not injured by the punctures; no hemorrhage into the pericardium. The spleen, liver, and kidneys are congested. There are abscesses in the medullary portion of both kidneys, and films show

TABLE I.

Time after inoculation.	Colonies per cc. of heart's blood.	Remarks.
1 min.	4,000,000	
9 "	100,000	
2 hrs.	100	Vomiting and diarrhea.
6 "	60	Drowsy.
25 "	200	Sluggish; has not eaten.
49 "	4,000	Lethargic; does not eat.
72 "	5,000	" hyperesthetic.
96 "	22,000	" "
120 "	3,000	Lying in cage; "
144 "	700	Hyperesthetic.
168 "	5	Comatose.
180 "	0	Found dead.

many diplococci and polymorphonuclear leukocytes. The subdural spaces of the brain and cord contain pus, and films show numerous pneumococci and polymorphonuclear leukocytes. The pneumococci are evenly distributed and are not being phagocyted.

In this experiment (Dog 1) we have a typical representative of a large percentage of the successfully infected dogs. The culture made 1 minute after the inoculation gave 4,000,000 colonies per cc. of blood. The pneumococci left the circulation rapidly and at the 6th hour 1 cc. of blood gave only 60 colonies. The blood cultures take a similar course in every instance and for convenience this phase of the infection will be designated as the initial drop. The initial drop was followed by a gradual increase in the number of bacteria in the blood and at the 96th hour 1 cc. gave 22,000 colonies. This proved to be the height of the blood infection and was followed by a second abrupt drop, the blood being sterile at the 180th hour. The secondary drop in this instance reminds one of the termination of pneumonia in man by lysis. The septicemia was evidently not the cause of death in this case, since at the time of death the heart's blood was sterile. On the 3rd day the dog became hyperesthetic. The hyperesthesia gradually gave way to paralysis of the extremities and this was followed by coma. Opisthotonos was also present. The autopsy revealed a severe purulent meningitis.

Dog 2.—Weight 6.5 kilos. 13 cc. of a bouillon culture of pneumococci were given intravenously. The record is given in Table II.

TABLE II.

Time after inoculation.	Colonies per cc. of heart's blood.	Remarks.
1 min.	7,250,000	
10 "	190,000	
7 hrs.	200	2 hrs. vomiting and diarrhea.
24 "	430	Sluggish; does not eat.
48 "	1,000	" " " "
72 "	6,000	Hypersensitive.
96 "	32,000	Definite symptoms of meningitis.
120 "	0	Lying in cage; cannot stand.
144 "	0	" " " " "
168 "	0	" " " " "
192 "	0	" " " " "
216 "	10	" " " " "
240 "	0	Eats; cannot stand. Gradually recovered.

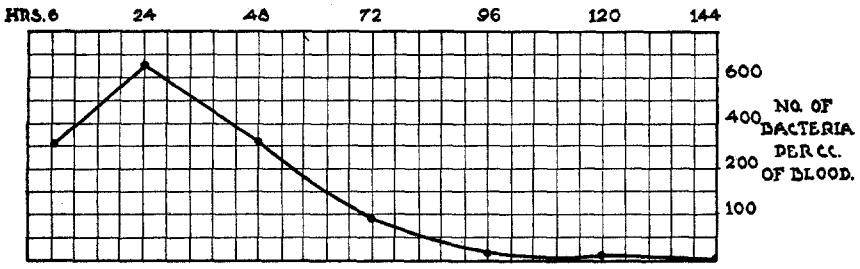
This dog differs from the first (No. 1) in that the secondary drop in the septicemia was of the nature of a crisis, going from 32,000 colonies per cc. of blood to sterility within 24 hours, with recovery. No relapse occurred in the septicemia, although a severe meningitis existed for several days. The pneumococci isolated on the 9th day grew in chains and were non-virulent (Fig. 1).

Dog 3.—Weight 7 kilos. 7 cc. of a bouillon culture of pneumococci were given intravenously. The record appears in Table III.

TABLE III.

Time after inoculation.	Colonies per cc. of heart's blood.	Remarks.
1 min.	3,800,000	
10 "	80,000	
6 hrs.	75	2 hrs. vomiting and diarrhea.
24 "	140	Does not eat.
48 "	100	Eats.
72 "	75	Lively.
96 "	0	"
120 "	0	No further symptoms.

In Table III (Text-fig. 1) is given the course of the bacteremia when no infection develops. The initial drop occurs as in other cases. There is a slight return from the 24th to the 48th hour, but, as a rule, the blood becomes sterile before the 5th day and no further symptoms develop.



TEXT-FIG. 1. Composite curve of ten dogs that did not become infected. They were given intravenously from 1 to 3 cc. of a bouillon culture of virulent pneumococci per kilo of body weight. The abscissæ represent in hours the time after the inoculation; the ordinates, the number of pneumococci per cc. of heart's blood.

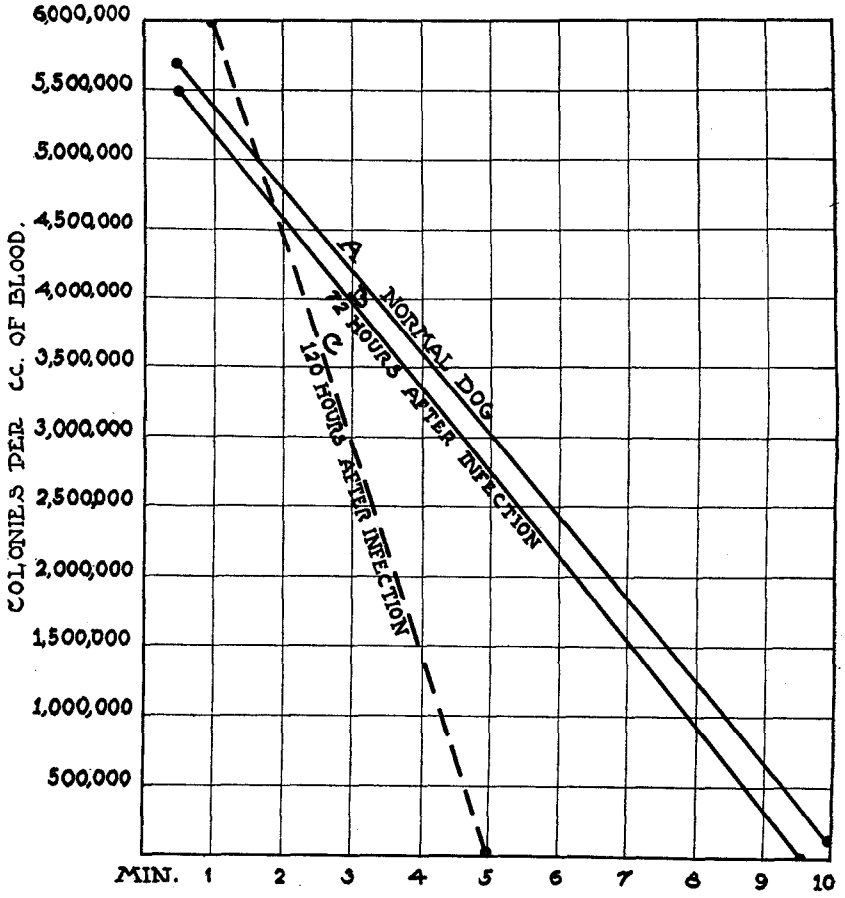
Dog 4.—Weight 6 kilos. 12 cc. of a bouillon culture of pneumococci were injected into the ear vein (Table IV).

TABLE IV.

Time after inoculation.	Colonies per cc. of heart's blood.	Remarks.
1 min.	6,500,000	
6 hrs.	300	Drowsy.
24 "	1,500	Sluggish.
48 "	7,000	"
72 "	10,000	"
96 "	20,000	Meningeal symptoms.
120 "	4,000	Very sick.
144 "	1,300	Cannot stand.
168 "	5,000	Legs paralyzed.
192 "	35,000	Comatose.
216 "	50,000	"
240 "	110,000	"
255 "	25,000	Found dead.

Autopsy.—A severe purulent meningitis.

This protocol (Dog 4) is given because of the relapse of the septicemia. It is possible that this was due to an invasion of the blood from the meningeal infection.



TEXT-FIG. 2. Curves A, B, and C represent the rate at which the pneumococci left the circulation of dogs at the time of infection and at 72 and 120 hours after the infection, respectively.

The curves (Text-figs. 1, 2, and 3) of the blood infections as presented in the protocols have three points of especial interest: the initial drop, the ascension of the septicemia, and the secondary drop. Several questions arise concerning the nature of the forces operating

during these three phases. First, why do the blood cultures fall so rapidly immediately after the injections are made? Why does the septicemia begin first after 24 or 48 hours instead of directly after the inoculations? Are natural antibodies present and do they destroy the mass of the pneumococci, then become bound or exhausted and permit the few bacteria that escaped destruction to multiply and produce the septicemia? Or, on the other hand, may the natural antibodies not be exhausted, the fittest organisms survive and later become immune to the forces that destroyed the mass? As to the secondary drop it may be assumed as a working hypothesis that the blood suddenly acquires new forces that destroy the pneumococci or remove them from the circulation. These points are considered individually in the following pages.

Initial Drop.

Observations made in following the course of typhoid bacilli when intravenously injected into normal rabbits[9] and the effect of immune serum on pneumococcic septicemia in rabbits[8] led us to suspect that the pneumococci are agglutinated in normal dogs immediately after reaching the circulation. Indeed, experiments proved this to be the case. Pneumococci from 100 to 150 cc. of bouillon culture were injected into the ear vein and specimens of blood were taken from the heart at short intervals from the 1st to the 10th minute and examined for clumps. The observations are shown in Table V and Figs. 2 and 3.

TABLE V.

Time of taking specimen of blood.	No. of free bacteria.	No. of clumps.
min.		
1	Many.	No clumps.
2	Fewer.	Several.
3	Still fewer.	Many.
4	" "	Fewer.
5	" "	Still fewer.
6	" "	" "
7	None.	" "
8	"	" "
9	"	None found.

[9] Bull, *J. Exp. Med.*, 1915, xxii, 475.

The dog died on the 4th day.

Autopsy.—Lungs hemorrhagic; pleurisy with effusion; many pneumococci in the fluid; kidneys hemorrhagic; abscesses in the medullæ; severe meningitis.

The character of the clumps here observed differs from those formed in rabbits treated with immune serum in that they are much less compact. The agglutination proceeds more slowly also. The degree of agglutination is, however, directly proportional to the rapidity with which the blood cultures decrease. By the cultural method it was demonstrated merely that the number of viable bacteria speedily decreases immediately after the inoculations. The microscopic observations demonstrated, moreover, that the reduction in the cultures is due to the absence of the bacteria and that it is not a question of viability. An examination of the organs gave further proof of this since clumps of the bacteria were readily found.

The initial drop, then, is due to the agglutination of the pneumococci within the circulation and to the accumulation of the clumps in the lungs, liver, spleen, and other organs.

Secondary Drop.

Since the initial drop is the result of an agglutination of the pneumococci, it was logical to suppose that the secondary drop was due to a similar process. Yet, in order to explain the removal of the bacteria which had invaded the blood despite the presence of the natural agglutinins, or possibly after they had been exhausted, it was necessary to assume either that newly acquired agglutinins appear or that the pneumococci suddenly became more susceptible to the previously existing agglutinins. Hence experiments were devised to test these points.

Specimens of blood were taken from a series of dogs and the dogs were then inoculated with pneumococci in the usual way. The sera obtained before the inoculation and specimens taken each succeeding day were tested *in vitro* for agglutinins and opsonins. Neither agglutinins nor opsonins could be thus demonstrated before the 6th or 7th day. This was 48 hours after the break in the septicemia, and hence the assumption that these antibodies were operative in the animals at the time of the crisis in the septicemia seemed not to be

justified. It was thought, however, that the test could be made successfully in the animal body by determining the extent of agglutination and rate of disappearance of pneumococci injected at the time of the crisis. Thus, it was found that agglutination is more pronounced and that the bacteria leave the circulation much more rapidly at this time than even in normal dogs (Text-fig. 2). Hence a wide disparity exists between the demonstration of certain antibodies *in vitro* and *in vivo*. Representative experiments of this type are given in the protocols of Dogs 5 and 6.

Dog 5.—Weight 4.75 kilos. 14 cc. of a bouillon culture of pneumococci were given intravenously. On the 5th day after the inoculation the pneumococci from 150 cc. of a bouillon culture were injected into the ear vein, and cultures and smears were made from the heart's blood at short intervals, from the 1st to the 10th minute after the inoculation. Cultures: 1 minute, 210,000,000 per cc. of blood; 9 minutes, 2,000,000 per cc. Films: 1 minute, many free diplococci and some clumps; 3 minutes, many clumps and few free bacteria.

Dog 6.—Weight 7.75 kilos. 18 cc. of a bouillon culture of pneumococci were given intravenously. On the 5th day 10 cc. of culture were injected into the ear vein and cultures were made from the heart's blood at 1 and 8 minutes respectively. Cultures: 1 minute, 7,840,000 colonies per cc. of blood; 8 minutes, 12,500.

By comparing the rapidity with which the bacteria leave the circulation of normal dogs with that of Dogs 5 and 6, it is readily seen that the disappearance is more abrupt and complete in the latter. In normal dogs about one-twentieth of the cocci injected were still present at the expiration of 10 minutes, while in the dogs injected on the 5th day of the infection only from $\frac{1}{200}$ to $\frac{1}{600}$ of the injected bacteria were present at the expiration of 8 minutes. Since the rate at which bacteria leave the circulation of an animal is directly proportional to the extent of agglutination, we have here corroborative evidence of the microscopic finding that pneumococci are much more actively agglutinated in infected dogs at the time of the crisis in the septicemia than in normal dogs.

The results of these experiments have two points of unusual interest. First, it is shown that bacterial antibodies are effective in the animal body before they can be demonstrated *in vitro*. Second, it is demonstrated that acquired antibodies are active at the time of a crisis in pneumococcic septicemia in dogs. These facts are doubly

interesting because they seem to shed light on the cause of the crisis in pneumonia in man. Acquired antibodies do not become demonstrable *in vitro* in the serum of pneumonia patients, as a rule, until some time after the crisis,[10] but in the light of the above findings one is justified in concluding that they are operative in the patients at the time of the crisis.

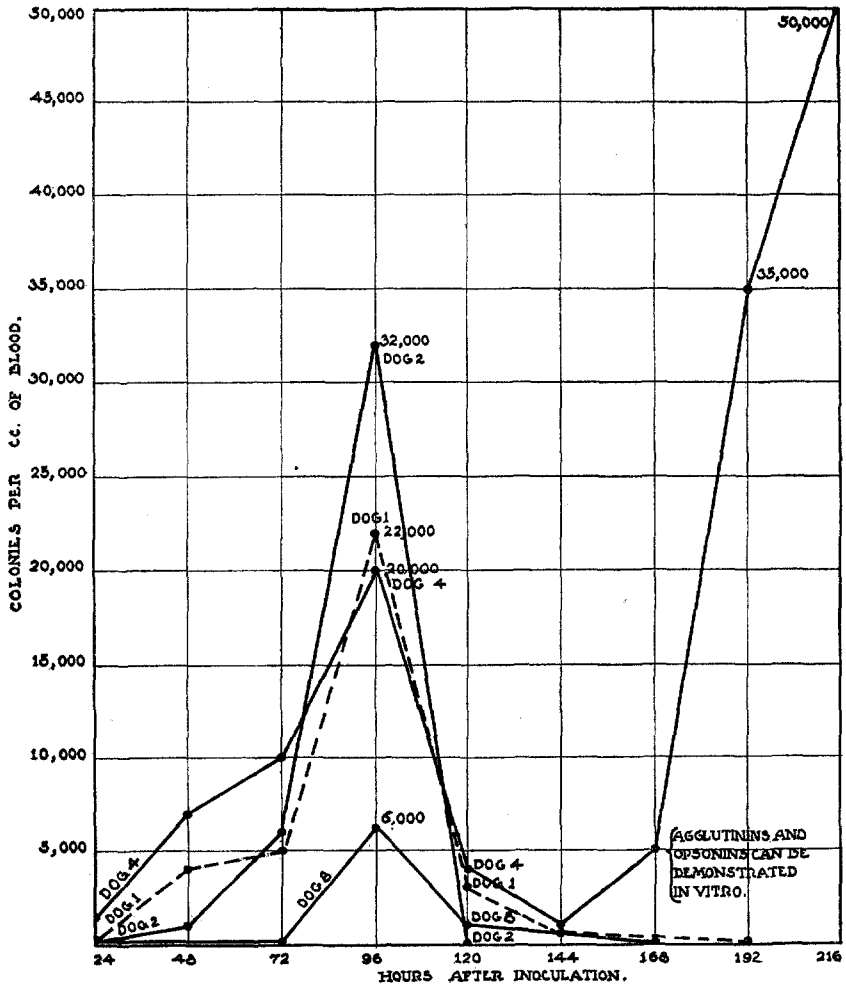
Ascension of the Septicemia.

After the initial drop the septicemia gradually increases for 48 hours and then rapidly rises to its apex (Text-fig. 3). Why a blood invasion is possible at this time, when the bacteria could not remain in the circulation at the time of the injection, is not immediately evident. Have the natural antibodies been exhausted or bound by the injected bacteria, or have the bacteria that escaped destruction become immune to the antibodies? It is possible that the septicemia is a resultant of two factors; a partial depression of the antibody activities and a lessened susceptibility on the part of the bacteria. The following experiments were made to study these points.

Bacteria.—Pneumococci were isolated from the heart's blood at each phase of the septicemia and kept on blood agar plates in the ice box until the infection had run its course. On the 6th day bouillon cultures were made from the different plates and the strains were tested for agglutination and opsonization with sera obtained from dogs on the 6th and 7th days of the infection. Thus it was found that the strains isolated as the septicemia was ascending were resistant to the action of the sera, while those isolated as the septicemia was declining were more readily opsonized and agglutinated than the original strain (Figs. 4 and 5). The resistant strains were both opsonized and agglutinated by a highly active immune horse serum, and the increased resistance disappeared after six transfers in bouillon.

Antibodies.—Dogs exhibiting a progressing septicemia were reinjected and the rate of disappearance of the newly injected pneumococci was determined.

[10] Dochez, A. R., *J. Exp. Med.*, 1912, xvi, 665.



TEXT-FIG. 3. Crisis in septicemia.

Dog 7.—Weight 8.5 kilos. 24 cc. of a bouillon culture of pneumococci were given intravenously. For blood cultures and reinjection see Table VI.

TABLE VI.

Time after inoculation.	Colonies per cc. of heart's blood.	Remarks.
1 min.	8,500,000	
6 hrs.	350	
24 "	600	
48 "	5,000	
		After the culture was made 8 cc. of culture were injected into the ear vein.
1 min. after reinjection.	3,500,000	
3 hrs. " "	3,000	
72 hrs.	14,500	
96 "	24,000	
120 "	Dog was found dead.	Meningitis.

The figures in Table VI show that even in the presence of a progressing septicemia freshly injected pneumococci rapidly leave the circulation (Text-fig. 2). Indeed, there were fewer bacteria in the heart's blood 3 hours after the second inoculation than immediately before. It was found that the number of pneumococci in the circulation 3 hours after the second injection was always in direct relation to the number present at the time the inoculation was made. As a rule, the cultures made at the expiration of 3 hours gave from 50 to 75 per cent as many colonies as those preceding the reinoculation. This held good although the dogs had infections ranging from 250 to 150,000 colonies per cc. of blood at the time they were reinoculated.

A question as to the identity of the residual pneumococci naturally arose. Are they the organisms of the septicemia, or a portion of the freshly injected culture? As already stated, cultures isolated during the ascension of the septicemia have been found to be resistant to the opsonins and agglutinins of weakly immune sera. Hence, if the residual pneumococci are the septicemic organisms, they should manifest this same resistance. This proved to be the case. Bouillon cultures were made with blood from the heart immediately before and 3 hours after the second inoculation and were tested on the following day for agglutination and opsonization with

serum taken from a dog on the 6th day after inoculation with pneumococci. The culture with which the dog was reinoculated was both opsonized and agglutinated, while the cultures obtained from the heart's blood immediately before and 3 hours after the inoculation were neither opsonized nor agglutinated.

An interpretation of the different phases of the blood infection is now possible. The initial drop is the result of the agglutination of the pneumococci in the circulation of the dogs and the accumulation of the clumps in the organs. After an incubation period of from 24 to 48 hours the pneumococci become resistant to the action of the agglutinins and possibly other antibodies and reinvade the blood stream. But before the septicemia can become fatal it is halted by the appearance of newly formed antibodies.

Severity of the Meningitis and Phagocytosis.—As previously stated, every fatal infection was accompanied by meningeal symptoms and a purulent pneumococcic meningitis was found at autopsy. A few dogs recovered after a prolonged attack of meningitis. Others, having lighter attacks and showing signs of improvement, were killed in order to study the phenomena that accompany improvement or recovery.

Films and sections from the brain and cord of the fatal cases showed large numbers of pneumococci and polymorphonuclear leukocytes. The pneumococci were evenly distributed and little or no phagocytosis had taken place. Similar preparations from dogs killed while in a state of improvement gave a different picture. The pneumococci were more or less collected into clumps and many leukocytes contained large numbers of bacteria (Figs. 6 and 7).

DISCUSSION.

The facts established in the above experiments are interesting when considered in connection with many obscure points in the epidemiology and course of infectious diseases. First, it has been shown that bacteria isolated at different stages of an infection vary in their susceptibility to specific antibodies and in their infecting power. This recalls the fact that many infectious diseases are more readily transmitted from person to person during the active phase of the dis-

ease than in early convalescence. Epidemics arise, probably, because the infectious agent is passed at short intervals from individual to individual during the early stage of the disease and, therefore, before the agent has been weakened by immunity responses on the part of the individual. As soon as early and frequent contacts are avoided by isolation, the agent becomes less infectious and the epidemic subsides.

An incubation period of about 48 hours always preceded the pneumococcic septicemia in the dogs, although millions of the bacteria were injected directly into the circulation. The experiments devised to explain this delay have shown clearly that this time is necessary for the pneumococci to adapt themselves to their new and adverse environment. When they had once become immune to the injurious antibodies that existed, a rapid multiplication occurred and symptoms of disease became manifest. And if these facts can be used to interpret the incubation period of infectious diseases in man, the logical conclusion is that it has a similar meaning there, for if bacteria find ideal conditions for multiplication on entering a new host, only a few hours will elapse between the time of infection and the appearance of symptoms of disease.

It may at first seem paradoxical that the pneumococci became more resistant to the action of antibodies in the early part of the infection and then later more susceptible. This is probably due to the nature and strength of the antibodies operative at the different phases of the infection. In the incubation period, only the natural antibodies are present, while in the decline of the septicemia acquired antibodies have appeared. Corroborative observations have been made *in vitro*. Feiler found that typhoid bacilli grown in fresh normal rabbit serum become more resistant to the bactericidal action of the serum,[11] while Stryker found that growth in highly immune horse serum renders pneumococci more agglutinable and less virulent.[12] Whether bacteria become weaker or stronger in the presence of antibodies depends, no doubt, upon the relation between the plasticity and adaptability of the bacteria and the strength of the antibodies.

[11] Feiler, M., *Z. Immunitätsforsch., Orig.*, 1916, xxiv, 411.
[12] Stryker, L. M., *J. Exp. Med.*, 1916, xxiv, 49.

The demonstration of the activity of newly acquired antibodies at the time of the crisis in the septicemia throws light upon the natural recovery from infections diseases in man. If the host can offer sufficient resistance to the invading bacteria to forestall a profound depression of the physiologic functions, or a fatal issue, until specific antibodies can be formed and mobilized, recovery will result. On the other hand, if the host offers little or no resistance to the bacteria at the time of infection and the incubation period is eliminated, the battle is lost before defensive antibodies can be formed. The latter condition is found in the case of pneumoccocic septicemia in rabbits and the former in pneumococcic septicemia in dogs.

The cases of meningitis offered an excellent opportunity to study the manner of the destruction of the bacteria in pneumococcic infections. Despite the fact that it has been demonstrated that agglutination, opsonization, and phagocytosis bring about the destruction of myriads of pneumococci in infected rabbits treated with immune serum,[13] it is still held that this has little or nothing to do with the destruction of the bacteria in cases of pneumonia in man.[14] One argument against the phagocytic theory is that phagocytosis is never found in the lungs of patients dying of pneumonia. What takes place in the cases that recover is, of course, undeterminable. The observations made on the acutely fatal and convalescent cases of pneumococcic meningitis in dogs indicate that the degree of phagocytosis is inversely proportional to the acuteness and severity of the infection. In the fatal cases there was very little phagocytosis, while in the convalescent cases the pneumococci were being actively phagocyted. The same condition may hold in pneumonia in man.

SUMMARY.

Intravenous inoculations of from 1 to 3 cc. per kilo of body weight of a bouillon culture of virulent pneumococci produce septicemia and meningitis in dogs.

The injected pneumococci leave the circulation rapidly, but begin to reinvade the blood from 24 to 48 hours later. The septicemia reaches its climax between the 4th and 5th days and then abruptly

[13] Bull, *J. Exp. Med.*, 1915, xxii, 466.

[14] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1916, xxiii, 61.

declines, the blood becoming sterile within from 1 to 3 days after the height of the septicemia is reached.

The initial disappearance of the pneumococci from the circulation has been found to be due to agglutination of the diplococci in the blood stream and accumulation of the clumps in the lungs, liver, spleen, etc.

If the dogs are reinoculated during the ascension of the septicemia, the injected diplococci leave the circulation as rapidly as in normal dogs. Cultures isolated in this stage of the infection, both before and from 3 to 4 hours after the reinoculation, are resistant to the agglutinins and opsonins of immune sera that agglutinate and opsonize the cultures with which the dogs were originally infected. Thus it follows that the pneumococci are able to reinvade the circulation because they have acquired a fastness to the existing antibodies and not because the antibodies have been bound or exhausted.

By reinoculating dogs at the time of the crisis in the septicemia it has been shown that the agglutination of the pneumococci is more rapid and complete and that the diplococci leave the circulation much more rapidly than in normal dogs. Hence acquired antibodies are operative within the animals at this time although they cannot be demonstrated *in vitro* until from 24 to 48 hours later.

Pneumococci isolated as the infection is subsiding are more susceptible to the action of immune sera than the original cultures injected.

It is probable that all the dogs would have survived the infection if a meningitis had not developed.

In the acutely fatal cases of meningitis few pneumococci are phagocyted, while in the milder and convalescent cases much phagocytosis occurs.

It is suggested that the incubation period of infectious diseases is due to the fact that the infecting agents must become adapted to the adverse conditions encountered in the newly infected host before they can multiply sufficiently to produce the symptoms of disease. It is further suggested that epidemics may arise because the infectious agent is passed from person to person in the ascending stage of the disease and thus enters new hosts in a state of maximum resistance to the natural antibodies of such individuals. When early contacts

are avoided, epidemics tend to subside because the infectious agent is weakened by the action of acquired antibodies during the period of convalescence.

EXPLANATION OF PLATE 6.

FIG. 1. Pneumococci isolated from Dog 2 on the 9th day of infection. The film was made from a bouillon culture.

FIG. 2. A film from the heart's blood of a dog 30 seconds after a suspension of pneumococci had been injected.

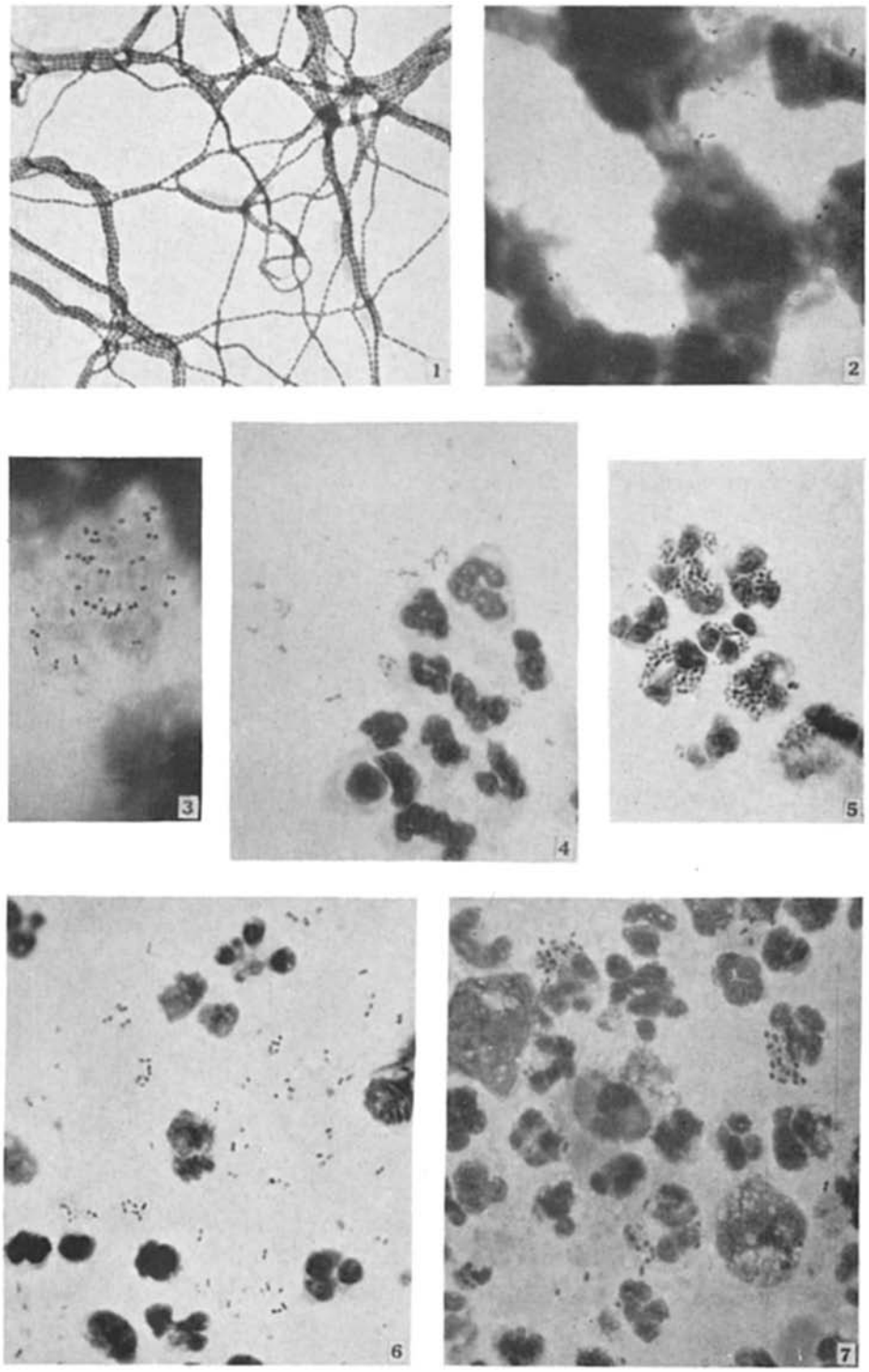
FIG. 3. A clump of pneumococci from the heart's blood of the same dog 2 minutes after the bacteria were injected.

FIG. 4. Pneumococci, immune dog serum, and guinea pig leukocytes. The bacteria were isolated from a dog 72 hours after it had been infected with pneumococci. The serum was obtained from a dog on the 7th day of a pneumococcic infection.

FIG. 5. The same as Fig. 4 except that the pneumococci were from the strain with which the above dog was infected.

FIG. 6. A film from the brain surface of a dog dying of an acute pneumococcic meningitis.

FIG. 7. A film from the brain surface of a dog in the convalescent stage of a pneumococcic meningitis.



(Bull: Immunity in Pneumococcus Infection.)