

A STUDY OF THE MECHANISM OF RECOVERY FROM EXPERIMENTAL PNEUMOCOCCUS INFECTION.

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In previous investigations of the nature of natural immunity to pneumococcus infection (1-3) evidence of a defense process common to both pneumococcus-resistant and susceptible animals was found. It was possible to demonstrate, by appropriate methods, pneumococidal activity in varying degrees with serum-leucocyte mixtures of the different animal species studied. The relatively marked pneumococcus-killing power of the naturally immune animals' blood was found to depend on the presence of opsonins capable of causing the phagocytosis and destruction of highly virulent pneumococci. The blood of susceptible animals lacked this property of influencing virulent strains but exhibited opsonic activity and destroying power for pneumococci of low virulence for the species. In relation to the total resisting capacity of the body, the degree of potential pneumococcus-killing energy resident in the blood suggests that it is an important if not the principal means of normal antipneumococcus defense. The question then arises as to whether recovery from pneumococcus infection is brought about by a similar mechanism or whether other and quite different forces are employed by the body in terminating the activity of pneumococci which have succeeded in overcoming the initial resistance of the animal. The results of a study of this problem by the same methods employed in the previous work constitute the subject of the present communication.

A review of the recent literature on immunity factors in recovery from experimental pneumococcus infection reveals several points of view which have arisen as a result of a diversity of experimental findings. Certain investigators, Bull (4), Tchistovitch (5), Tudoranu (6), and others, believe that recovery is

brought about by the elaboration of specific immune substances, agglutinins, opsonins, or protective bodies which promote the destruction of the disease producing microorganisms by the leucocytes. Other workers take an entirely opposite view, namely that recovery is quite independent of these manifestations of humoral immunity. Singer and Adler (7) eliminate humoral immunity completely from the mechanism and hold that a specific immune change occurs in the cells of the reticulo-endothelial system by which they are enabled to engulf and destroy the invading pneumococci. Wadsworth (8) considers that the production of humoral immune substances is an important element in the recovery process but is of the opinion that pneumococcus immunity is chiefly antitoxic in nature. The majority of investigators, however, have come to the conclusion that the elaboration of antibodies of the bacteriotropic or protective type constitutes a part of the process of acquired resistance but that there are other undetermined factors which play a contributory, and at times a preponderating rôle.

It has been found repeatedly that active immunity may occur in the absence of demonstrable serum immune properties. Cecil and Blake (9) failed to find any constancy in the appearance of protective substances in the blood of monkeys recovering from pneumococcus pneumonia or any correlation between the degree of active immunity of the animal and the passive immune properties of its serum. Wright (10), studying experimental pneumococcus infection in rabbits, observed that the development of opsonic and protective properties was irregular and uncertain and did not appear to parallel the acquisition of active immunity. Other authors have made analagous observations.

This inconstancy in the finding of immune bodies in the serum of animals with acquired resistance appears to depend to some extent on the type of invading pneumococcus. While Cecil and Blake found protective substances occasionally after infection with Types I and II, none were observed to occur in the serum after pneumonia due to *Pneumococcus* Types III and IV. Differences in antibody response to the several pneumococcus types are brought out more clearly by active immunization. In contrast to the comparative ease and regularity with which it is possible to produce immune substances against Types I and II in the different animal species is the uncertainty of antibody response following immunization with Type III. In a recent study Tillett (11) found that only four of twenty-eight rabbits immunized with Type III pneumococcus showed type-specific antibodies in the serum. Tudoranu, however, considers that the difference between immune sera Types I and II and serum Type III is one of antibody concentration only and if enough serum of a Type III immunized or recovered animal is used, specific protection can be demonstrated against the homologous pneumococcus. Felton (12) has thrown further light on this subject by finding that in antipneumococcus serum or antibody solution there is an inhibitory or antagonistic substance which can be eliminated by fractional precipitation. He was able to show that a Type III antiserum thus treated had a much augmented protective power.

The appearance of serum immune bodies appears to be conditioned even more by the kind of animal employed in the study, than by the type of pneumococcus used. Those authors who have found opsonic activity constantly in the serum of animals recovering from pneumococcus infection (Bull and Tchistovitch) worked with a highly resistant species, the dog. Wadsworth, studying the problem, failed to find increased phagocytic power in dogs' blood regularly, but he never found any evidence of this reaction in infected rabbits. The majority of those workers whose findings have led them to question or reject the probable causal relationship between the occurrence of humoral immunity and recovery have worked with animals highly susceptible to pneumococcus, chiefly rabbits and monkeys.

Among the experimental data of the above authors on pneumococcus infection in susceptible animals one finds occasionally suggestions that there may be some relationship between the degree of active immunity and the presence of demonstrable immune properties. Wright, for example, found evidences of humoral immunity, especially the pneumococidal power of the blood, most constant and most marked at a time when the resistance of the animal (rabbit) was greatest. More recently Stillman (13) made the observation that rabbits exposed repeatedly to sprayings with *Pneumococcus* Type I developed protective properties in their serum, the percentage incidence of which increased progressively with the number of sprayings. Since the per cent mortality among the rabbits diminished with the number of exposures to pneumococci it is highly probable that the surviving animals had developed an increased resistance.

In taking up the present work, namely an attempt to determine the part played by humoral immunity in the mechanism of recovery from experimental pneumococcus infection, it seemed wisest to investigate first the reaction of resistant animals since they have been found to yield more constant demonstrable evidence of antibody production; then in the light of the findings which might result from such a study to proceed to observations on a susceptible species.

Methods.

Disease Production.—Cats and rabbits were infected, for the most part by the intrapleural injection of actively growing and abundant broth cultures of pneumococci. A few animals were injected intraperitoneally. The organism used principally was a Type I pneumococcus originally isolated from the blood of a case of lobar pneumonia. After passage through twelve cats its virulence for these animals was found to lie between 0.005 cc. and 0.1 cc. broth culture. Rabbits were killed in the amounts of 0.000,000,1 cc. and mice with 0.000,000,01 cc. Passage through a cat was repeated every month or two during the course of the work. A Type II and a somewhat less virulent Type I pneumococcus were

also used in rabbits. The former killed in doses of 0.1 cc. to 0.01 cc., the latter with 0.000,01 cc. to 0.000,001 cc. broth culture.

Disease Course.—The cats which recovered ran a febrile course usually from 4 to 5 days terminating often with an abrupt fall in temperature. Occasionally the fever persisted for a longer period. A well marked loss in weight was a constant accompaniment. This usually ceased with the fall in temperature but in some animals there was a slight further weight loss followed by a slow subsequent gain, in spite of their appearing otherwise well. The course of infection in rabbits was much more irregular and fluctuations in temperature were found to be less significant than loss in weight. The experimental animals were observed from weeks to months.

Pathology.—The lesions produced in cats by intrapleural inoculation were found to vary widely. Autopsy after fatal termination of the infection showed, in the majority of instances, empyema of one or both sides often with pericarditis and sometimes peritonitis. In only one case was lobar pneumonia observed. Several animals showed no gross pathology, but blood cultures were positive. Some of the cats were killed during and immediately after recovery. Except in one instance in which organized fibrin was observed over the pericardium, no gross lesions were found in these animals. Cats dying after intraperitoneal injection showed peritonitis but no pleuritis. In the rabbits which died, a diffuse peritonitis and pleuritis were found. Lobar pneumonia was not seen. Microscopical studies of the tissues were not made except to verify the presence of pneumonia. Secondary infection with streptococci or Gram-negative bacilli supervening either during the course of infection or after recovery was not uncommon.

Serum.—The test serum was allowed to separate in the cold, then cleared by centrifugation and immediately inactivated at 56° for $\frac{1}{2}$ hour. All specimens from a single animal, kept on ice in sealed tubes, were tested at one time. The dilutions indicated in the tables represent final dilution in the serum-leucocyte mixture.

Pneumococidal Tests.—The serum of the infected animals was tested for its power to promote pneumococcus killing by adding it to normal rabbit serum-leucocyte mixtures seeded with virulent pneumococci. The technique was the same as that described in a previous paper in the study of this property of specific antipneumococcus serum (14). The infected animals' leucocytes were not used because it was not possible to obtain sufficient cells for the tests without withdrawing injuriously large quantities of blood and in the experiments with cats, normal leucocytes could not be employed since it would have been necessary to add normal active cat serum which mixture possesses pneumococcus-killing power. Heating the serum annuls this action. By using the serum-leucocyte mixture of a normal susceptible animal which of itself has no pneumococidal action, any degree of this property occurring can be attributed directly to the effect of the added infected animal's serum. The amount of pneumococcus suspension

with which the serum-leucocytes were seeded was chosen arbitrarily as containing a sufficient number of pneumococci to produce abundant macroscopic growth in 15 to 18 hours but still few enough to permit effective action of the immune serum in high dilution.¹

Opsonic and Agglutination Tests.—The serum was tested for opsonins and agglutinins according to the technique described in a preceding paper (3). The ratio of serum to pneumococcus suspension employed was 20:1.

EXPERIMENTAL.

Cats.

Development of Pneumococidal Promoting Properties in the Serum Following Infection and Their Relation to Active Immunity.

It was found constantly that the serum of cats recovering from experimentally induced pneumococcus infection possessed the power to promote destruction of pneumococci in rabbit serum-leucocyte mixtures analogous to that caused by the presence of specific anti-pneumococcus serum. This property or substance was heat-stable and could be demonstrated in high dilutions of the serum. The method of testing such sera is shown in Table I in which are given the details of the tests made on the serum specimens of Cat 1, Text-fig. 1, secured before and after infection. It is seen that the presence of inactivated normal cats' serum had no detectable retarding effect on the growth of pneumococci in rabbits' serum and leucocytes even in dilution of 1:10 (lower dilutions could not be used on account of the disturbing effect of cytotoxic action), whereas the serum of the same cat obtained after recovery from a well marked infection, conferred pneumococcus-killing power on these mixtures in dilution as high as 1:5120. The immune horse serum tested at the same time as a control on the activity of the rabbit serum and leucocytes showed a potency ten times as great.

Following recovery the cats were found to be resistant to many times the fatal dose of pneumococci.

¹ In a previous paper (14) it was found that there exists a quantitative relationship between the concentration of immune serum present and the number of pneumococci destroyed by the serum and leucocytes.

Appearance of Serum Immune Properties in Relation to Temperature and Blood Invasion.

Tests on samples of blood taken frequently during the course of infection showed that in those animals destined to recover, serum immune properties appeared about the 4th day of the disease (Text-figs.

TABLE I.

Determination of Pneumococcal Promoting Power of Cat's Serum before and after Pneumococcus Infection.

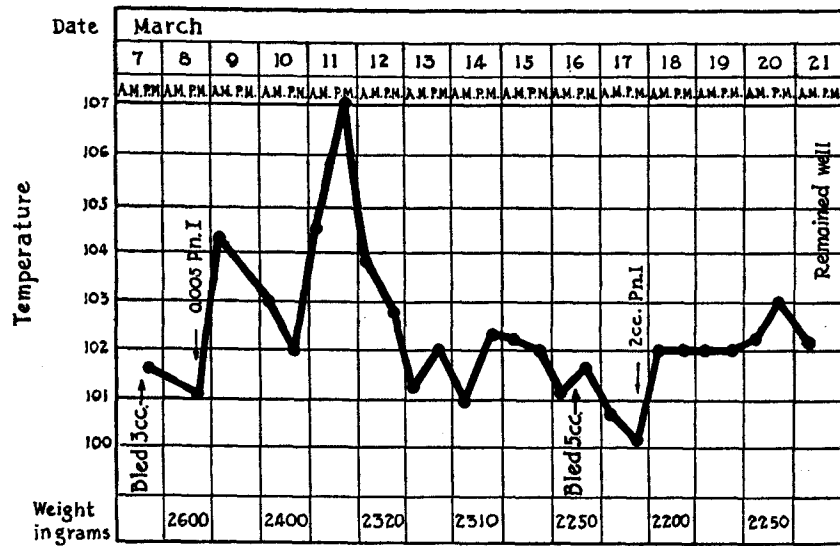
Normal rabbit serum 0.2 cc. + rabbit leucocyte suspension 0.1 cc. + diluted cat serum 0.1 cc. + pneumococcus suspension 0.1 cc.

	Amount of standard pneumococcus suspension	Dilutions of cat serum	Growth as shown by color change at hrs.*				Pneumococci in stained film at 72 hrs.
			15	18	24	48	
	<i>cc.</i>						
Cat's serum before inoculation	0.000001	1:10	++++				+
"	"	1:20	++++				+
"	"	1:40	++++				+
"	"	1:80	++++				+
Cat's serum after recovery	"	1:320	0	0	0	0	0
"	"	1:640	0	0	0	0	0
"	"	1:1280	0	0	0	0	0
"	"	1:2560	0	0	0	0	0
"	"	1:5120	0	0	0	0	0
"	"	1:10240	0	0	++		+
		Dilutions of immune horse serum					
Controls with immune horse serum	"	1:6400	0	0	0	0	0
"	"	1:12800	0	0	0	0	0
"	"	1:25600	0	0	0	0	0
"	"	1:51200	0	0	0	0	0
"	"	1:102400	0	0	+++		+
Controls with normal rabbit serum and leucocytes only	"		++	+++			+
"	0.0000001		+++	++++			+
"	"		+	+++			+
"	"		+++	++++			+
"	"		+++	++++			+

* Degree of methemoglobin formation.

2, 5, and 6). In two cases they were demonstrable on the 3rd day. The titer of the pneumococcal promoting substances rose abruptly and usually reached its highest point within 48 hours where it tended to remain comparatively stationary for several days before beginning a gradual drop. Coincident with the appearance of immune bodies in the serum there occurred in most instances a sharp drop in temperature, although often it did not reach normal until several days later.

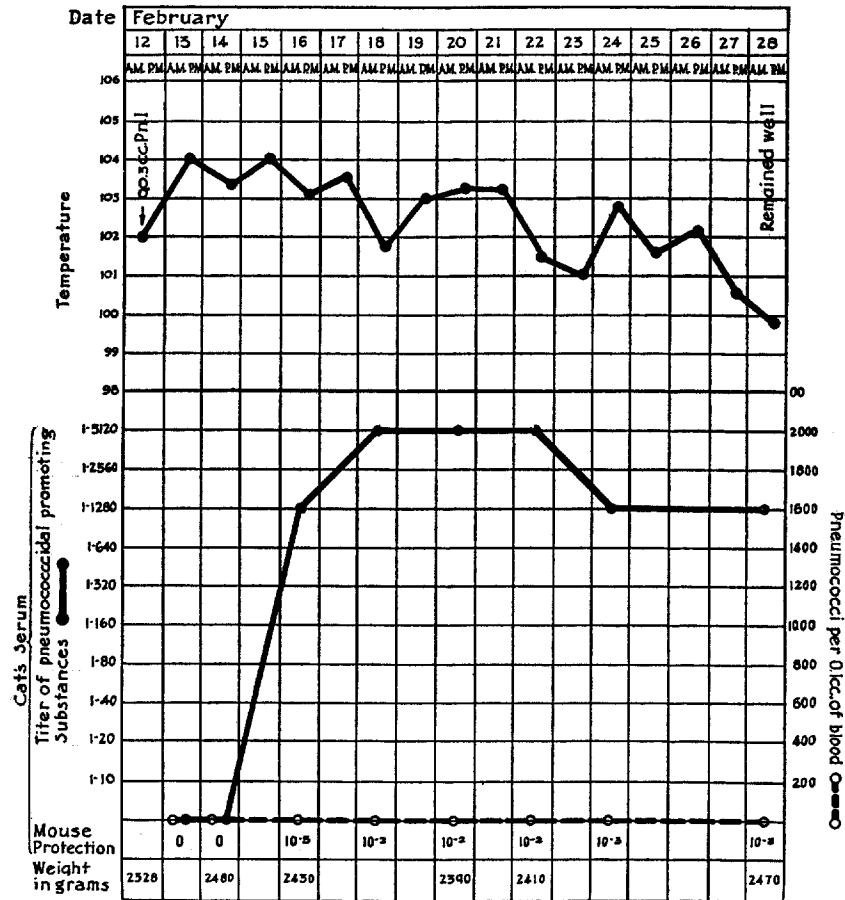
Blood invasion which was present in the majority of the infected cats and often to an intense degree, terminated abruptly with the



TEXT-FIG. 1. Cat 1. Experimental pneumococcus infection following the intrapleural injection of 0.005 cc. of Pneumococcus Type I broth culture.

appearance of pneumococcal promoting substances in the blood (Text-figs. 3, 5, and 6). In most instances this immune property was not demonstrated until the blood was sterile, but occasionally as shown by Cat 5, Text-fig. 5, both antipneumococcus substances and pneumococci were found to be free in the blood stream at the same time. It is possible that had blood specimens been taken with sufficient frequency this transient simultaneous presence of circulating

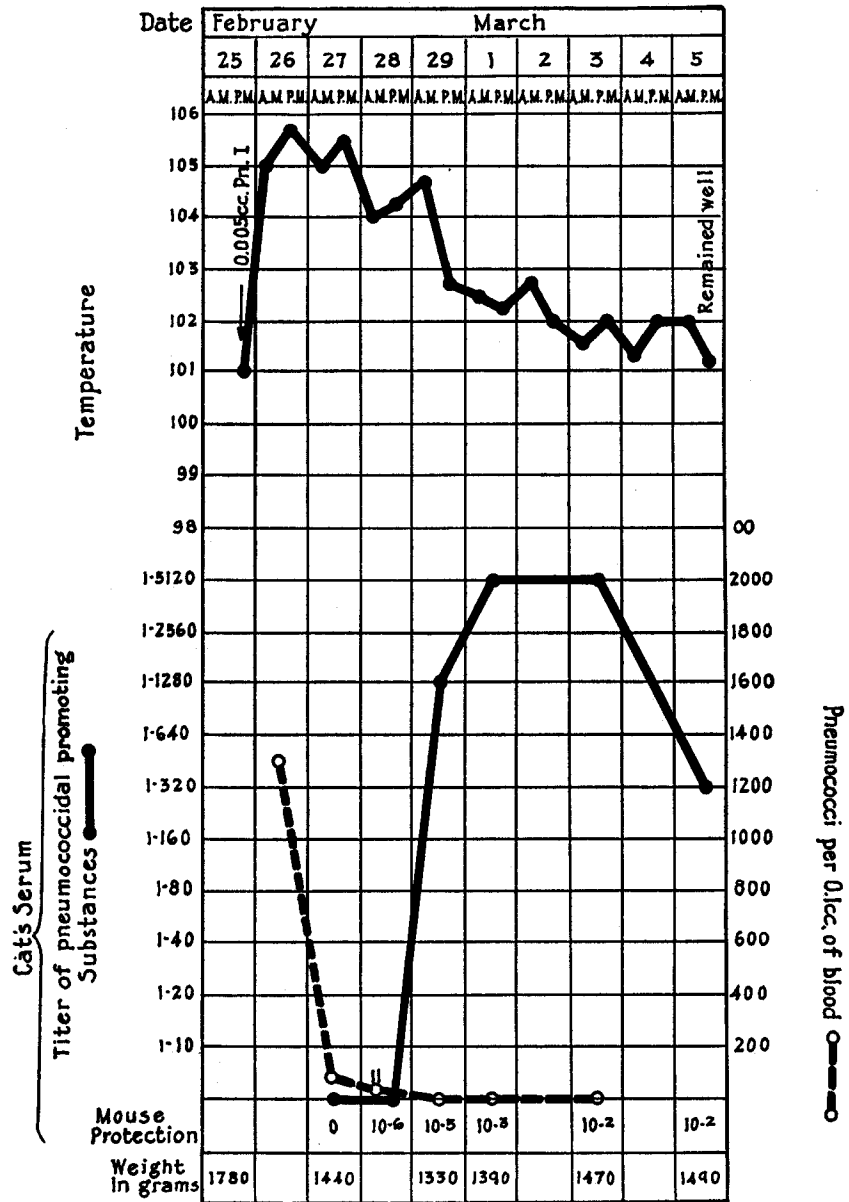
antigen and antibody would have been observed much more frequently or perhaps regularly. The constant and marked decrease in the number of colonies shown by blood plates during the 24 to 48 hours



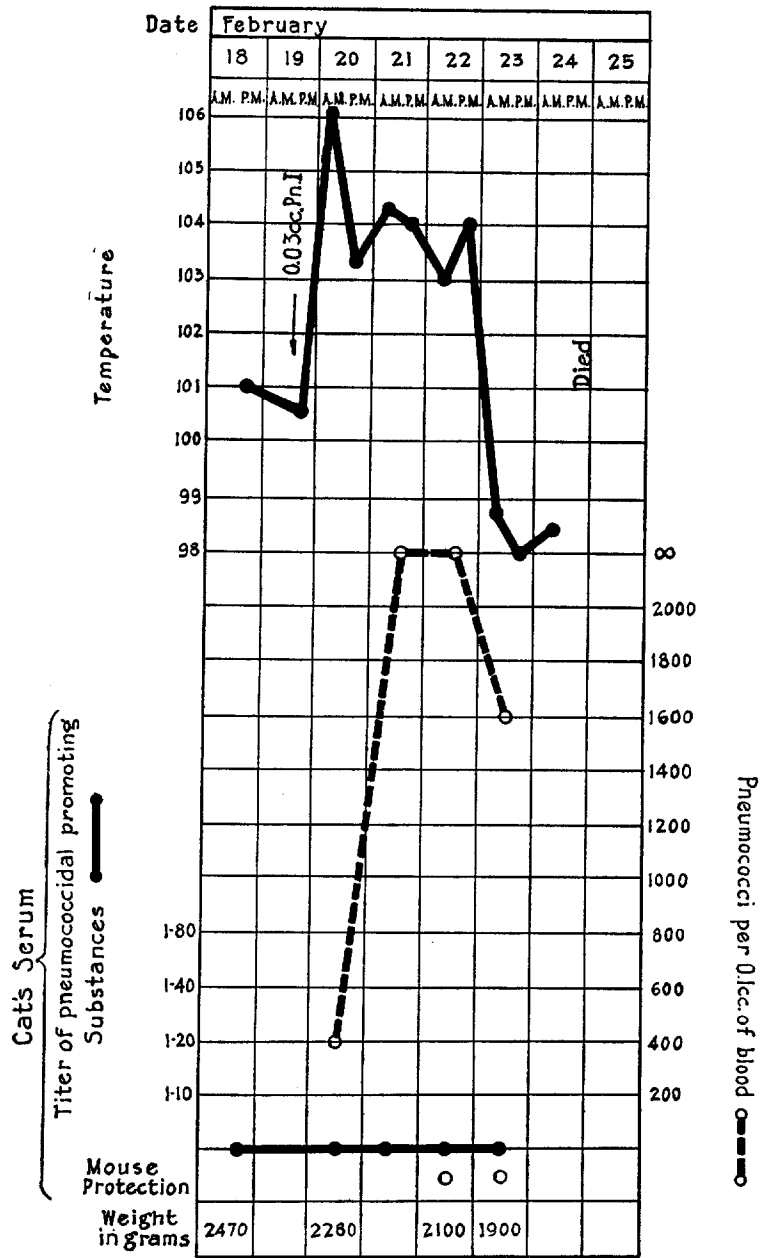
TEXT-FIG. 2. Cat 2. Experimental pneumococcus infection following the intrapleural injection of 0.03 cc. broth culture, Pneumococcus Type I.

preceding the appearance of immune substances in the blood, suggests that the elaboration of these bodies begins early in the disease.

All those cats in which the disease went on to a fatal termination

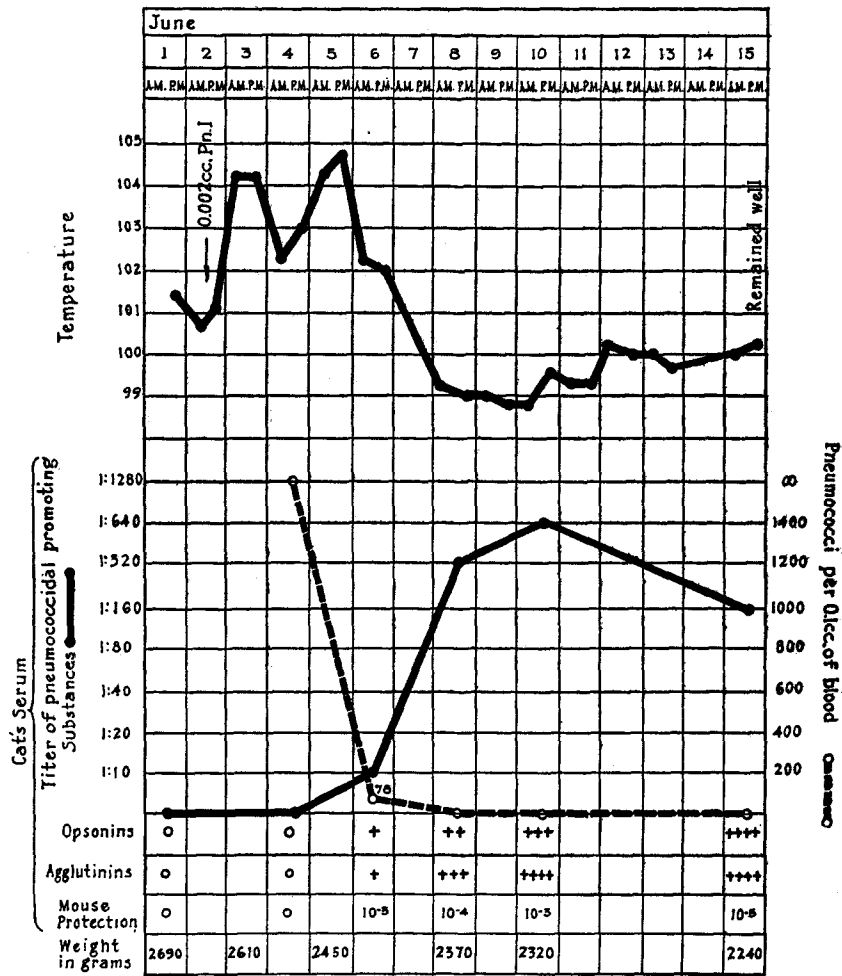


TEXT-FIG. 3. Cat 3. Experimental pneumococcus infection following the intrapleural injection of 0.005 cc. broth culture, Pneumococcus Type I.



TEXT-FIG. 4. Cat 4. Experimental pneumococcus infection following the intrapleural injection of 0.03 cc. broth culture, Pneumococcus Type I.

failed to develop demonstrable serum immune properties and showed a persistent marked bacteriemia (Text-figs. 4 and 7).



TEXT-FIG. 5. Cat 5. Experimental pneumococcus infection following the intraperitoneal injection of 0.002 cc. broth culture, Pneumococcus Type I.

Character of Immune Bodies Occurring at Recovery.

In a number of instances serum specimens were also tested for their opsonic, agglutinative, and mouse protective actions. The results of

one such complete study are shown in Text-fig. 5. A very close parallelism was found to exist between the appearance of these three well recognized evidences of antipneumococcus reaction and the pneumococcal promoting power of the serum. With very few exceptions they all appeared at the same time and increased together although not necessarily in a strictly quantitative relationship. In two cases mouse protective properties² were demonstrable before pneumococcal promoting power became apparent. Text-fig. 3 shows one such instance. A repetition of the test gave the same result. And in one other cat the serum showed slight opsonic activity the day preceding the detection of the other evidences of immune change. The absence of exact parallelism between the appearance and rates of increase in intensity of these several reactions affords, however, no objection to the supposition that they represent different manifestations of one immune response since the various methods employed to bring them out are not comparable quantitatively. During the initial period of recovery when the concentration of immune substances was low, frequently neither agglutinative nor opsonic activity could be demonstrated with equal parts of serum and pneumococcus suspension but could be brought out clearly when a ratio of twenty parts serum to one part pneumococcus suspension was used.

The animals which died failed to show at any time during the course of the disease any of the above studied serum immune properties.

Specificity of Serum Immunity.

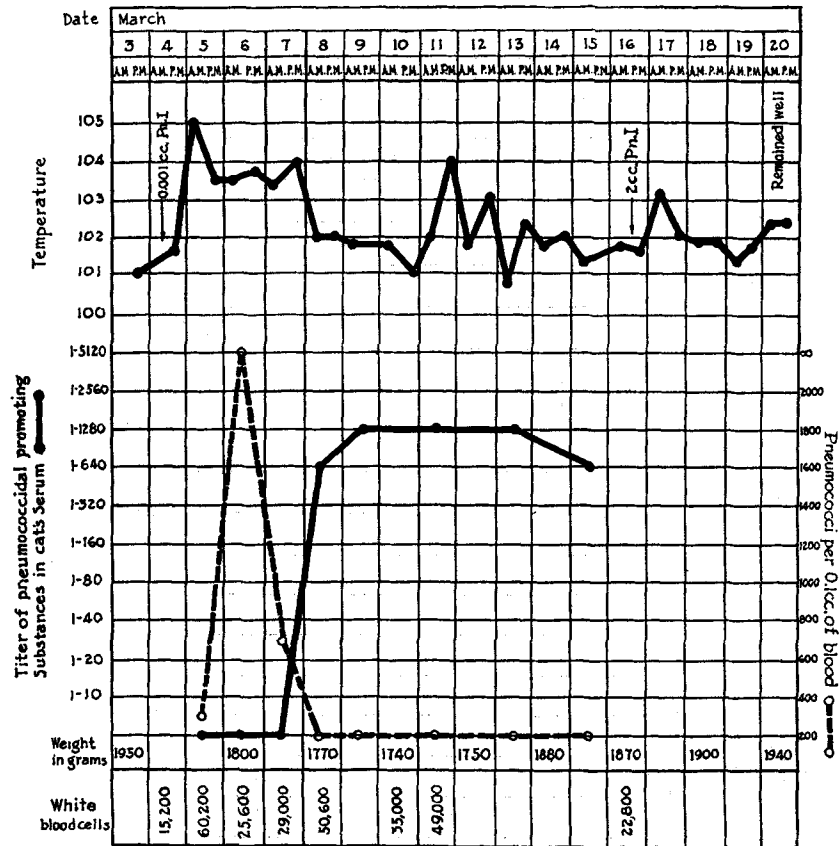
Tests carried out to determine the specificity of the immune response in the serum of recovered cats showed that such serum was strictly type-specific as to pneumococcal promoting, agglutinative, and opsonic properties.

Relation of Leucocyte Count to Course of Disease.

Estimation of the number of circulating white blood cells made at frequent intervals during the experimental disease, failed to reveal any constant difference in leucocyte response between the animals recovering from infection and those which succumbed. After an

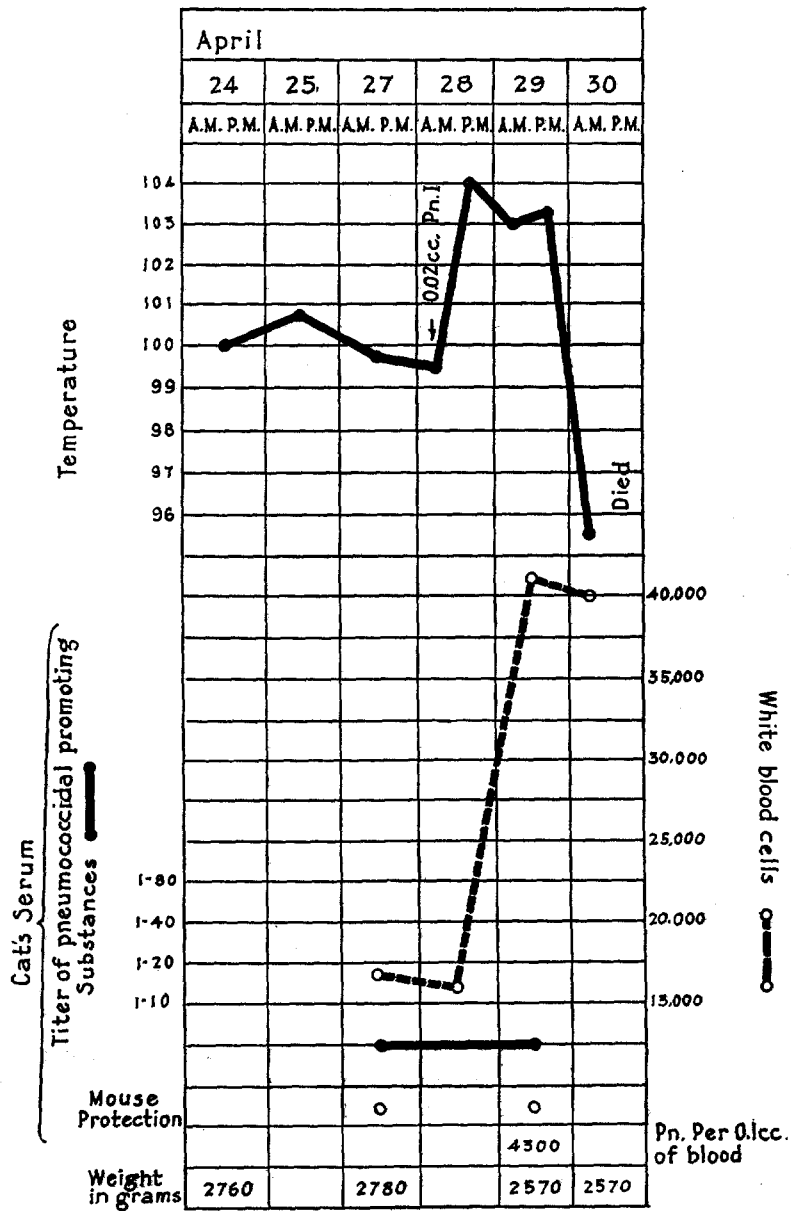
²The figures recorded on the charts indicate the dilution of culture against which 0.2 cc. of the animal's serum protected.

initial rise within the first 24 to 48 hours from a normal count of 10,000-15,000, to 30,000-50,000, the number of leucocytes usually diminished gradually during the course of the disease. In a few instances a secondary rise was noted just preceding recovery. In sev-



TEXT-FIG. 6. Cat 6. Experimental pneumococcus infection following the intrapleural injection of 0.001 cc. broth culture, Pneumococcus Type I. Reinoculated after recovery with 2 cc. broth culture.

eral cats both with fatal and non-fatal outcome the number of white blood cells increased only slightly. It was of interest to observe repeatedly an unusually high leucocyte count accompanying fatal



TEXT-FIG. 7. Cat 7. Experimental pneumococcus infection following the intraperitoneal injection of 0.02 cc. broth culture, Pneumococcus Type I.

infection (see chart of Cat 7, Text-fig. 7). Another cat (chart not exhibited) showed a count rising from 33,000 on the 1st day to 50,000 on the 4th day when death occurred. Still another died on the 8th day with the white blood cells at 42,000. Counts as high as 85,000 were observed within 2 or 3 days of death. In certain of these animals showing a terminal high white count empyema was found, but in others there was no evidence of complications.³

It is evident from these observations that the course of pneumococcus infection in the cat is not determined primarily by the number of circulating leucocytes. In the absence of detectable serum immunity the leucocytes, no matter in how great numbers present, seem to have no inhibiting effect on either blood invasion or progression of the disease. Cat 6, Text-fig. 6, showed a rapidly increasing number of pneumococci in the blood in spite of a leucocyte count of 60,000, and it was not until humoral immune bodies appeared that blood invasion ceased. It was noted repeatedly that with immune substances present in the blood a moderate increase only in the number of leucocytes appeared to be compatible with rapid and complete recovery.

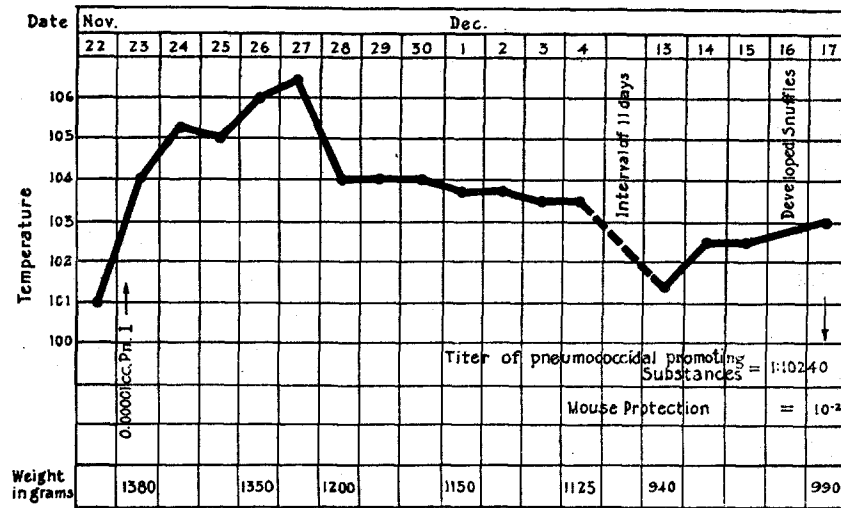
While the scope of this work did not include a complete study of the duration of active and passive immunity following infection, observations were made on three cats after a lapse of 2 months. Just prior to reinoculation with 10 to 20 times the killing dose of pneumococci two of the cats showed a low titer of pneumococcal promoting substances in the serum, the third showed none. All three animals reacted with a marked rise in temperature and weight loss but recovered in 3 to 4 days. In striking contrast to the behavior of these animals is the absence of any appreciable reaction observed in cats reinoculated soon after recovery from infection when the concentration of serum immune substances was at a high level (see charts, Text-figs. 1 and 6). These were two of the cats used for the tests 2 months later.

Rabbits.

Attempts to study in rabbits the type of experimental pneumococcus infection observed in cats were found to be attended by many

³ Furthermore the development of empyema was not always accompanied by a rise in the number of leucocytes.

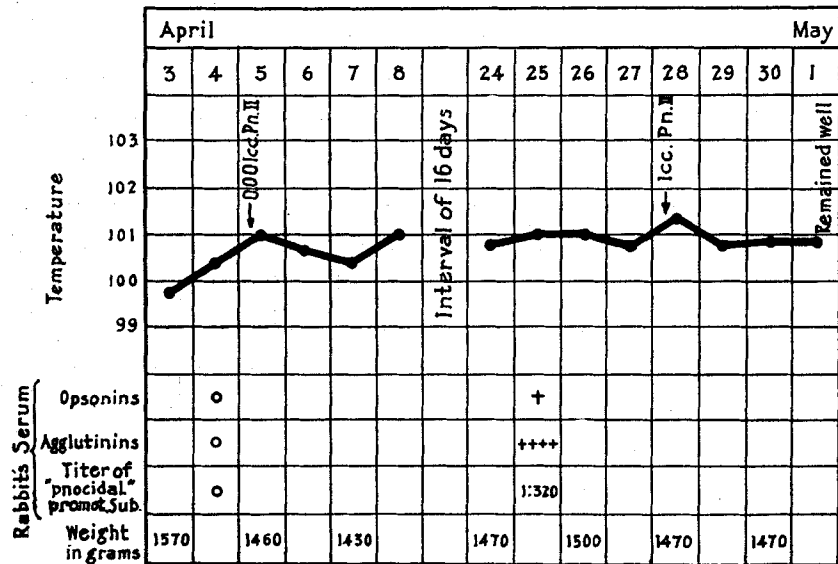
difficulties on account of the wide individual variations in susceptibility which rabbits manifest towards the pneumococcus. A great majority of the animals inoculated, either died or failed to show evidence of disease. Furthermore, withdrawal of blood in any quantity during the stage of active infection had very disturbing and often disastrous effects. From a very large number of trials a few satisfactory observations were secured but these were by no means as complete as those made on cats.



TEXT-FIG. 8. Rabbit 1. Experimental pneumococcus infection following the intrapleural injection of 0.000,01 cc. broth culture, Pneumococcus Type I.

The main findings in cats as regards the occurrence of immune substances in the blood, were confirmed in the rabbit. Following recovery from experimental pneumococcus infection, there appeared in the rabbit's serum pneumococcal promoting substances which were shown to be associated with the acquisition of greatly increased pneumococcus resistance (Text-figs. 8, 9, and 10). Likewise opsonic, agglutinative, and mouse protective properties were demonstrable in the serum at the same time. The intensity of the immune response appeared to be fully as great in rabbits as in cats when similar experimental conditions obtained.

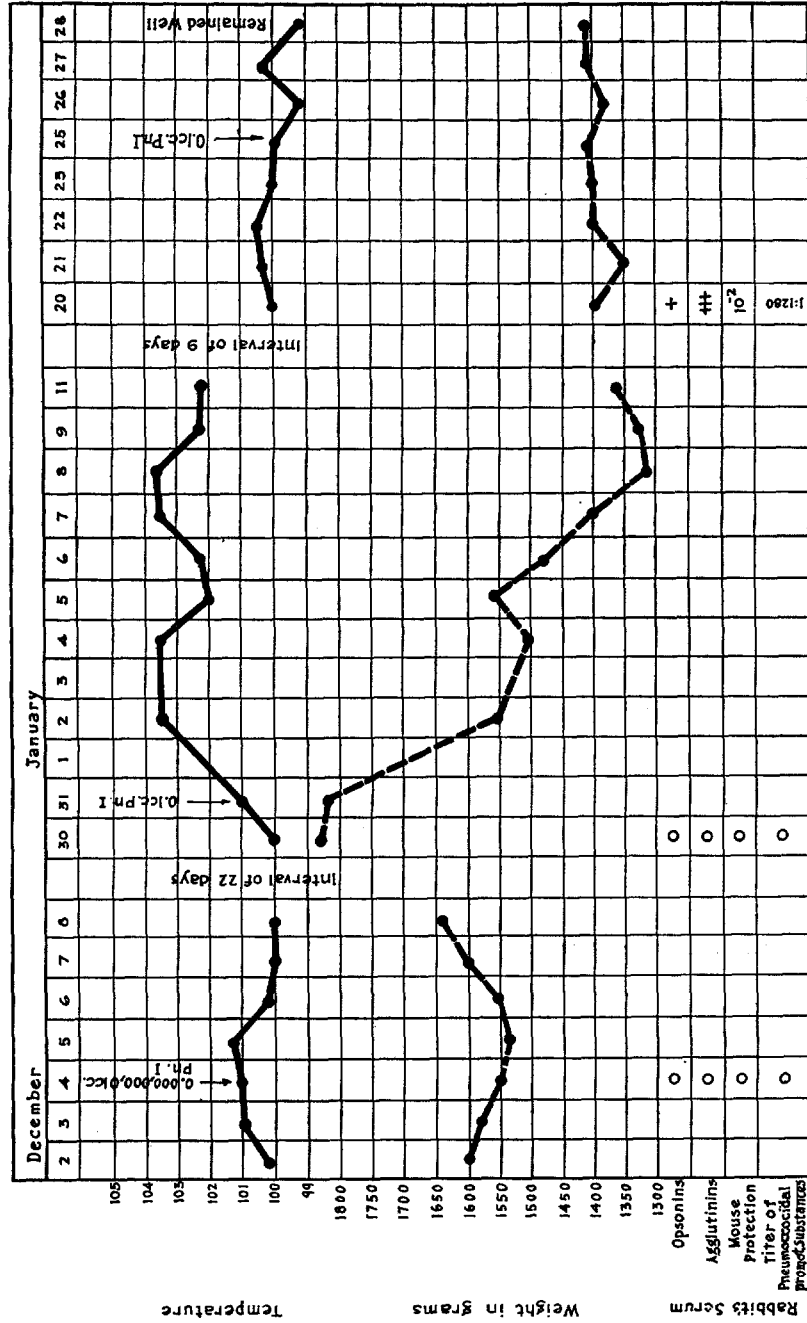
The charts of the three rabbits exhibited exemplify several types of reaction to pneumococcus infection which differ considerably one from the other. Rabbit 1, Text-fig. 8, received a small dose of a virulent strain and after a severe and prolonged disease course showed a high concentration of serum immune substances.⁴ Rabbit 2 after a much larger dose of a pneumococcus of considerably less virulence, gave scarcely any evidence of disease but showed a well defined though low titer of pneumococidal promoting power in the serum. With



TEXT-FIG. 9. Rabbit 2. Experimental pneumococcus infection following the intraperitoneal injection of 0.001 cc. broth culture, Pneumococcus Type II. Reinoculation after recovery with 1 cc. broth culture.

Rabbit 3, on the other hand, the injection of a minute number of highly virulent pneumococci, produced no appreciable effect and no resulting detectable serum immunity but the animal was found to have developed a very greatly increased resistance. While Rabbit 2 with demonstrable serum immune properties showed no reaction

⁴ Further observations were prevented by the development of snuffles which progressed slowly to a fatal termination.



TEXT-FIG. 10. Rabbit 3. Experimental pneumococcus infection following the intraperitoneal injections of 0.000,000,01 cc. broth culture, Pneumococcus Type I, on December 4, and 0.1 cc. on December 31. Reinjection of 0.1 cc. on January 25.

following the injection of many times the lethal dose of culture, Rabbit 3, lacking such serum properties, developed a severe though not fatal disease after reinoculation. The subsequent appearance of immune substances in the serum of Rabbit 3 indicated a further increase in resistance as revealed by the lack of any detectable reaction to a second reinoculation of the same size.

Observations on the leucocyte count were not made in the rabbits chiefly on account of the uncertainty of the outcome of the experimental disease. Neither were the rabbits retested for duration of their active or passive immunity.

DISCUSSION.

The most noteworthy result of this work is the constancy of the findings. In the resistant animal recovery was always marked by the development of well defined humoral immune properties which failed to occur in fatal infections. In the susceptible animal studied (the rabbit) similar immune reactions were found although they could not be related so closely to the period of recovery. The observations in cats tend to amplify the studies of certain previous investigations on experimental pneumococcus infection in resistant animals. Bull (4), who has made the most detailed report on this subject, was not able to demonstrate agglutinins and opsonins in the serum of dogs until 24 to 48 hours after the temperature had fallen, but he found indirect evidence of immune body action *in vivo*, at the time of crisis. Whether the success obtained in our experiments in demonstrating the coincidence of the appearance of serum immune substances with recovery was due entirely to the methods employed or to the particular experimental animal used remains to be determined.

Our findings in the rabbit do not agree altogether with those of other investigators. The results obtained in the study of Rabbit 3, Text-fig. 10, offer a possible explanation for at least some of these previously observed irregularities in the appearance of immune substances in susceptible animals. This animal, as a result of an exceedingly small injection of a highly virulent pneumococcus developed a marked degree of active immunity but showed no evidence of passive immune properties in its serum. Is this to be attributed to the presence of immune substances in such low concentration that they could

not be detected in the test-tube or mouse, or were the body cells only sensitized and ready to react more energetically to further antigenic stimulus, or was this state of resistance due to another factor? The finding of a relatively high concentration of serum immune substances following the severe infection produced by the second inoculation suggests the first or second possibility. Another factor which may introduce variations in the demonstrability of humoral immune bodies is possible differences among the susceptible animal species in respect to excess elaboration of antipneumococcus substances. Cecil and Blake (15), for example, found only very occasionally slight traces of immune properties in the serum of monkeys tested 2 to 3 weeks following experimental pneumococcus pneumonia with all four types.⁵ It seems not improbable that certain non-resistant species, as the monkey, may elaborate specific antibodies in only slight excess of the concentration needed to combat the invading microorganism and that this detectable excess disappears rapidly after recovery. Dochez (16) observed in certain cases the rapid disappearance of protective substances from the serum of patients after recovery from lobar pneumonia. We have also noted, following lobar pneumonia, the complete disappearance within 10 days of serum immune substances which were present at the time of crisis in considerable concentration.⁶

The chief significance of our observations on the occurrence of antipneumococcus immune substances in the cat would seem to lie in the time relation of their appearance to the termination of infection and the nature of their demonstrable action. The fact that substances capable of promoting a marked degree of pneumococcal action appeared in the serum coincidentally with the amelioration of the disease process as shown by drop in temperature, and cessation of blood invasion which was followed by continued progress to recovery, makes it seem probable that the development of humoral immunity bears a causal relationship to the mechanism of increased antipneumococcus defense. A perfectly valid criticism of this assumption is that we have tested only the animals' serum and that we have no

⁵ That the monkey shows a well marked antibody production under certain conditions was found by Cecil and Blake (15) in their studies in vaccination with virulent living pneumococci.

⁶ This finding will be reported in a subsequent study on lobar pneumonia.

evidence that the serum of the recovering animal promotes the killing of pneumococci in the cat's body as it does in the test-tube with normal leucocytes. It is true that we have made no observations on the phagocytic and intracellular digestive activity of the leucocytes during experimental pneumococcus disease. The studies of other workers (17, 18, 19), however, on the activity of the leucocytes during pneumonia, while not in entire agreement with each other, indicate that ordinarily their function is little if any diminished, but in infections of marked severity there may be some depression especially of the intracellular digestive power. This phase of the problem requires further investigation.

The essential change which occurs in the serum of the cat recovering from pneumococcus infection would seem to be the elaboration of a heat-stable body or bodies whose chief antibacterial manifestations are opsonic and agglutinative activity. Power to precipitate the soluble substance undoubtedly is also present since the immune change is specific. That all these reactions are brought to bear on the pneumococcus growing in the animal body seems probable. Whether these several functions of the immune serum assume the same relative importance in the test-tube serum-leucocyte mixture is open to question, since conditions of pneumococcus growth *in vivo* differ in some respects from those *in vitro*. Possibly the precipitative action plays a less important rôle in the serum-leucocyte tests than in the body where the products of pneumococcus growth are present in greater concentration. Again, agglutination may play a more prominent part *in vivo*, especially in freeing the blood stream from organisms. However, the fact that the potency of an immune serum to produce pneumococcus killing in the serum-leucocyte mixtures parallels very closely its protective power in the mouse's body (14), suggests that its chief function is the same in both environments. Since phagocytosis and intracellular digestion are promoted to a marked degree by the presence of antipneumococcus serum and constitutes the only means of pneumococcus killing by the blood elements which we have been able to demonstrate, we are led to infer that opsonic action in its entirety probably constitutes the most important property of such a serum.

The question then arises whether granting the possibility of de-

struction of pneumococci in the body by the above indicated means, will account for the degree of pneumococcus killing that occurs during recovery from infection. This question cannot be answered definitely since we have no means of estimating the number of organisms in the infected animal, but it is possible to estimate in a general quantitative way the killing power of immune blood. As shown in Table I, the immune horse serum is ten times as potent as the recovered cat serum. By repeated tests we have found that a 1 to 500 dilution of immune serum of this potency when added to the serum and leucocytes contained in 0.5 cc. normal blood is capable of causing the destruction of 10^{-3} of the standard pneumococcus suspension which amount contains approximately 1,000,000 pneumococci (14). With a serum one-tenth this potency we might expect that it would produce the same action in a dilution of 1 to 50, since the action of immune serum under these conditions has been found to be quantitative in nature. A cat weighing 2000 gm. would have a total blood volume of about 100 cc. and if each 0.5 cc. of blood could destroy 1,000,000 pneumococci the total amount disposed of by 100 cc. would be 200,000,000. But in making this estimate we are limiting the destruction to the normal number of circulating leucocytes. The serum is capable of sensitizing perhaps ten times or more as many organisms as can be cared for by the normal number of circulating white blood cells and it is likely, as shown by the work of others which will be discussed further on, that certain of the fixed tissue cells also take part in the phagocytosis under these conditions. Even if these figures, which are only approximate, err considerably on the side of too high an estimate they do show a relatively enormous killing potentiality in the serum and cells of the pneumococcus immune animal, and it is probable that this process occurs much more effectively and continuously in the body than in the test-tube.

The relation of the naturally immune state to these changes which have been found to take place during infection in the cat, is by no means clear. Assuming that the normal resistance of this animal depends chiefly on the presence of circulating antipneumococcus opsonins, it would seem probable that a pneumococcus sufficiently virulent to establish itself in a free cavity of the body must be able to

neutralize the normal immune substances.⁷ What happens next we can only surmise. This process of neutralization may take place rather slowly or it may be that the body has reserves of normal defense forces that can be called into action to retard the growth of the pneumococci until the development of the acquired immune reaction occurs. Wright considers that increased resistance may begin within a few hours after the introduction of the microorganisms and we also have reason to believe that the processes of acquired immunity are initiated relatively early in the disease course. The newly operating anti-pneumococcus opsonins detectable at recovery appear to differ in nature from the normal opsonins but we have as yet insufficient criteria to be sure of this. Our observations in a previous paper (3) on the relative heat resistance of normal pig opsonins raises the question as to the safety of relying entirely on this test for the differentiation of immune from normal opsonins. At any rate the pneumococidal action induced by the recovering cat serum in a rabbit serum-leucocyte mixture appears to be the same as that caused by the serum and leucocytes of the normal cat, but whether the pneumococcus-killing process which takes place in the animal successfully resisting infection by implanted pneumococci is the same as that employed by the diseased animal is open to question. Information concerning the type of cell engaged in phagocytosis and intracellular digestion of pneumococci in naturally resistant animals is far from complete. The evidence at hand, however, suggests that of the circulating leucocytes, the polymorphonuclears play the chief rôle in this process. The large monocytes have also been shown to be actively phagocytic for pneumococci. Observations in animals with acquired immunity indicate that a variety of cells take part in the disposal of pneumococcus.

Wright, studying the fate of pneumococci injected into rabbits immunized against *Pneumococcus* Type I found that in the lung the monocytes were fully as actively phagocytic as the polymorphonuclear leucocytes and that in the liver certain of the fixed tissue cells of the reticulo-endothelium engulfed large numbers of organisms. Singer

⁷ Bull (4) regards the early stages of disease as a period of adaption of the microorganism to the existing antibodies rather than to the exhaustion of these substances.

and Adler ascribe phagocytosis in both the immune and normal animal entirely to the reticulo-endothelium. However, they did not demonstrate this type of phagocytosis as occurring in the normal animal. Tudoranu found that in the aleuronat peritoneal exudate of rabbits immunized with *Pneumococcus* Type III the homologous organisms were phagocytosed almost entirely by the monocytes. The experiments of Winternitz and Kline (20) and also Wright in which the number of leucocytes were greatly reduced by benzene injections without appreciably diminishing the blood-clearing power of the immune animal, indicate that cells other than the leucocytes are active in the pneumococcus-destroying processes of the immune body. While all these experimental observations have been made in a susceptible animal, the rabbit, the same process may well occur in relatively resistant animals with acquired immunity and, were it possible to make observations in the normal animal body as easily as in the immune animal, cells, other than the leucocytes, might be found to be active in the destruction of pneumococcus. That the Kupffer cells of the liver in normal resistant animals are capable of taking up pneumococci seems quite probable but it is difficult to estimate the importance of this process in the normal defense mechanism against natural infection since we know practically nothing of the penetration of pneumococci into the blood stream except during disease.⁸

The observations on rabbits recorded in this study provide by themselves only indirect evidence as to the part played by humoral immunity in the rabbit's recovery from pneumococcus infection. But taken in conjunction with the findings in cats, they assume much greater significance. However, the fact should not be overlooked that acquired resistance in this animal has been shown to be present in the absence of demonstrable serum immunity and although certain plausible explanations for such absence can be made, there exists the possibility of other unknown immune factors.

It should be stressed that our experiments both with normal and infected animals have been carried on almost entirely with pneumococci of Types I and II. That the blood of pneumococcus-resistant

⁸ Kyes demonstration of the marked phagocytic power of the Kupffer cells of the pigeon for pneumococci suggests that these cells in the normal mammal may possess a similar function.

animals possesses the power to destroy *Pneumococcus* Type III (1) and that it possesses normal opsonins against this type (21), has been shown. But no experiments on infection with Type III pneumococci nor studies on normal resistance of susceptible animals against these organisms have been made. Tillett (22) has recently published a study which might be interpreted to indicate that natural and acquired immunity to *Pneumococcus* Type III may be of a different order than that against the other two fixed types. He found that the resistance of normal rabbits to relatively enormous intravenous doses of an encapsulated Type III pneumococcus was not associated with demonstrable antibodies in the serum and that little evidence of phagocytosis could be detected in the blood. Phagocytosis by the reticulo-endothelial system was not investigated. The most significant phase of Tillett's work deals with the production of active immunity to Type III which he finds to be not only not associated with demonstrable immune substances but to be non-specific. He considers that the normal rabbit possesses a mechanism for inflicting injury on the capsule of the Type III pneumococcus and that active immunity probably consists in an increase in this normal function.

In the light of our present incomplete knowledge it is not possible to draw any general conclusions concerning the processes underlying recovery from experimental pneumococcus infection. Until the whole field has been studied with the care that has been devoted to certain areas, the interrelationship of the component parts will probably not be revealed. However, the constant occurrence of phenomena observed in the study of certain aspects of the problem justify our drawing tentative inferences limited always to the conditions under which the observations were made. Thus the evidence at hand would indicate that recovery from experimental infection with *Pneumococcus* Types I and II in cats and rabbits is brought about largely by the elaboration of specific antipneumococcus serum substances whose chief function is to make possible phagocytosis and intracellular digestion of the invading pneumococci. That the evidence for this assumption is much clearer in cats than in rabbits is granted. Furthermore drawing the above conclusion does not exclude the possibility of other forces aiding in the process. There may be another factor in acquired immunity and certain experimental findings suggest its

existence, which plays a part perhaps relatively more important in one type of animal than another and possibly varying in its activity with the different types of pneumococci. That other pneumococcus-resistant animals react in a manner similar to the cat seems probable in view of observations of other investigators and because of the fact that these animals show the same type of normal protective mechanism. Inferences concerning the recovery processes of pneumococcus-susceptible animals other than the rabbit are less safe because of the great diversity of findings by different workers. However, the fact that humoral immune substances have been detected in these animals with acquired resistance, suggests that this form of immunity plays some part in their recovery from pneumococcus infection.

SUMMARY.

A study was made of the blood of cats and rabbits during experimental pneumococcus infection with a view to ascertaining the relationship of acquired immune properties to the mechanism of recovery. Observations were directed chiefly towards the detection of pneumococidal promoting substances, but the other manifestations of anti-pneumococcus reaction were studied as well. It was found constantly that the serum of animals recovering from infection possessed the power to promote the destruction of highly virulent pneumococci in rabbit serum-leucocyte mixtures which mixtures of themselves have no growth inhibitory action. Furthermore, the presence of this serum immunity was associated with a marked increase in acquired resistance to the pneumococcus. In cats which were studied in the most detail the pneumococidal promoting power of the serum as well as the opsonic, agglutinative, and mouse protective activities became demonstrable at the time of recovery and their appearance in the serum always marked the termination of blood invasion. These immune reactions were found to be type-specific. The animals which succumbed failed to develop detectable serum immune properties and showed persistent blood invasion. The degree of leucocytosis did not appear to bear any constant relation to the outcome of the disease. The significance of these findings is discussed.

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