EXPERIMENTAL PNEUMONIA IN GUINEA PIGS.

I. The Effect of Certain Toxic Autolysates of Pneumococci.

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Plates 18 and 19.

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Much work has already been carried out on the experimental production of pneumococcus pneumonia in animals. Important publications on the subject are those of Wadsworth (1) on the production of pneumonia in rabbits; of Lamar and Meltzer (2) and Meltzer and Wollstein (3) in dogs; of Cecil and Blake (4) in monkeys; and of Stillman and Stillman and Branch in mice (5–7).

It is logical to suppose that in order to produce pneumococcus pneumonia this organism must be made to grow in the lung. It has, however, been the experience of all workers that, in the case of the smaller laboratory animals—rabbits, guinea pigs, or mice—pneumococci will not infect the lung tissue save in exceptional instances, no matter how they are injected. Thus Wadsworth found that rabbits injected intratracheally with virulent or non-virulent pneumococci either failed to become infected or died with pneumococcus septicemia, but without pneumonic lesions. Stillman had the same experience in his attempt to produce pneumonia in rabbits by the inhalation method. In the case of mice also Stillman found that virulent pneumococci, though inhaled into the lower respiratory tract, disappeared within a few hours without calling forth a pneumonic reaction. Even in alcohol-intoxicated mice in which the pneumococci persisted in the lung for a longer period than in unintoxicated mice and in which fatal septicemia often followed, localization with production of pneumonia was rarely observed.

Wadsworth's negative results in attempting to produce pneumonia

in normal rabbits led him to repeat the experiments in systemically or locally predisposed rabbits. In this he was unsuccessful as was Stillman in similar experiments in mice. However, by provoking a partial immunity in rabbits, Wadsworth did succeed in regularly causing pneumonic lesions and recently Stillman has also succeeded in producing lobar pneumonia in partially immunized alcoholized mice exposed to an atmosphere of virulent pneumococci.

The obvious conclusion that emerges from this work on the production of pneumococcus pneumonia in the smaller laboratory animals is that under ordinary conditions, the pneumococci will not grow in the lungs of these animals and without such growth pneumonia is not produced. If this be true, one may imagine that in pneumococcus pneumonia in animals, and in man, there is a hypothetical substance or substances, perhaps produced in the growth or autolysis of the pneumococci, which by its action upon the lung tissues or fluids enables the pneumococcus to establish itself and to multiply, and in so doing to cause pneumonia.

In a previous communication (8) it was shown that Berkefeld filtrates of certain anaerobically produced autolysates of Pneumococcus I or Pneumococcus II cause necrosis when injected intracutaneously into guinea pigs. In the course of this work it was discovered that these autolysates when injected intratracheally were highly toxic, and that when introduced in this manner together with living pneumococci, the growth of the organisms in the lung became established. From these observations it seemed possible that such toxic filtrates might contain the hypothetical poisonous substances referred to above, and this report gives the experiments which were conducted in the study of this problem.

The experiments here reported refer (1) to the effect of toxic autolysate alone upon the lungs of guinea pigs, (2) to the effect of diluted toxin added to living pneumococci, in establishing the growth of the organism in the lungs. In this second group of experiments, controls were given of equivalent doses of (*a*) toxic autolysate alone, and (*b*) living pneumococci without autolysate.

We will discuss the biological and immunological properties of the lung-toxic substance or substances and compare these properties with those of the necrotizing poison in the autolysates in a later publication.

EXPERIMENTAL.

Intratracheal Injection of Toxic Autolysates Alone.

Preparation of Toxic Autolysates.—The method used in preparing the toxic autolysates was the same as that previously described (8).

Virulent Pneumococcus Type I or Type II was grown on double strength veal infusion broth containing 4 per cent Witte peptone. After 18 to 24 hours growth, and before any appreciable autolysis had taken place, the broth cultures were chilled and then centrifuged at high speed. After centrifuging the tubes were chilled, the supernatant fluid pipetted off carefully, and the pneumococci taken up in a quantity of freshly boiled and chilled broth equal to that of the combined sediment and supernatant fluid remaining. For the pneumococcus sediment from 100 cc. of broth culture, there should be in all approximately 1.5 cc. of fluid. The pH of the pneumococcus suspensions was brought to 7 or 7.2 and cultures made to be sure that only pneumococci were present in the preparation. Sometimes 0.25 per cent phenol was added to the preparations. The suspensions were distributed into narrow test-tubes, which were then chilled for at least 30 minutes. After this, any bubbles present on the surface of the suspensions were gotten rid of with a hot platinum loop and heavy vaseline seals added to all of the tubes. The tubes were left at room temperature in the dark at 22–24°C. for 2 to 5 days and then placed in the ice box. Before filtering, the autolysates were centrifuged, iced, the seals opened, and the clear supernatant fluid passed through a well iced Berkefeld filter. This filtrate contains the poison which is highly toxic when injected intratracheally. The filtrate was kept in the ice box under vaseline seal until used. It was necessary to keep the preparations chilled when they were exposed to the air, otherwise they became oxidized and the toxicity disappeared.

(*A*) *Results of Intratracheal Inoculation of Undiluted Toxic Autolysates Alone.*

It was found that strong necrotizing filtrates invariably produced marked symptoms followed by death when injected intratracheally in 0.2 cc. amounts in guinea pigs of from 180 to 300 gm. weight. The toxicity for the skin and for the lungs appeared to run parallel; that is, a filtrate which produced marked necrosis when injected intracutaneously was also extremely toxic when injected intratracheally into guinea pigs, while a filtrate of weak necrotizing activity caused only slight symptoms when injected intratracheally and the guinea pigs usually survived. Although there were individual differences in the reaction of guinea pigs even of the same weight after the injection of

a strong toxin, 0.2 cc. of the undiluted poison usually killed them in less than 3 hours, and, with three exceptions, in less than 24 hours. Guinea pigs almost invariably survived the intratracheal injection of strong toxin diluted 1 to 2 or 1 to 4 with broth.

1. Guinea Pigs Dying within 1 to 2 Hours after Injection.

Symptoms and Pathology—Within 5 minutes of the intratracheal injection of the toxic autolysates the guinea pigs showed marked dyspnea which continued until death. In the pigs which died in less than 6 hours, tonic and clonic muscular contractions and a bloody serous nasal discharge were noted before death. At autopsy, the lungs of the guinea pigs which died in less than 6 hours were greatly distended, very heavy, and mottled deep pink throughout. The cut section of these lungs centrally was extremely wet, deep red, and apparently airless.

Histological Findings.—Eight guinea pigs dying within 1 to 2 hours after injection were studied. All of these showed severe and definite pulmonary lesions: intense congestion, profuse alveolar hemorrhages, edema, stasis of leucocytes in the alveolar capillaries, and the presence of sparse numbers of emigrated leucocytes in the alveoli. The peribronchial tissues also were edematous, and loosely infiltrated with wandering cells. Fibrin was not conspicuous.

2. Guinea Pigs Surviving for 18 to 72 Hours after Injection.

In this group, $\frac{2}{3}$ to $\frac{3}{4}$ of both lungs was found at autopsy distended, heavy, deep red, and consolidated. There was a varying amount of normal tissue at the margins of some of the lobes. The cut surface of the red portions appeared completely solid. This group included animals injected with weak autolysates or with strong autolysates diluted 1 to 2 or 1 to 4 with broth and killed with ether at varying intervals after the injection. At autopsy, the appearance of the lung was similar to that described above save that the consolidated areas were concentrated in the hilum regions. It should be emphasized that the color of the pneumonic areas in all the animals injected with toxic autolysate alone—and over 100 have been examined—always was deep red and never even tinged with gray.

Histological Findings.—Fourteen animals of this group were studied. All showed patchy but quite extensive areas of pneumonic consolidation. The alveolar exudate in the earlier stages consisted of well preserved polymorphonuclear leucocytes, numerous red blood cells, and occasional large mononuclears. Fibrin was not abundant. There was swelling of the alveolar cells, necrosis in some areas of the alveolar wall with occasional formation of hyaline membranes. Edema of the peribronchial tissues and alveoli was less marked than in animals dying in the first few hours and massive hemorrhages were not present.

In animals killed after the 2nd day there were still pronounced pneumonic lesions, the ductuli alveolares were dilated and inflamed, and small pneumonic patches were scattered through the tissues; edema was still quite marked.

On the 3rd day the lesions showed evidence of regression, leucocytes were less numerous and undergoing fragmentation and autolysis. There was swelling and active proliferation (mitoses) of alveolar epithelium and capillary endothelium. In one animal the peribronchial lymphoid tissue appeared hyperplastic.

Results from Intratracheal Injections of the Following Preparations in 0.2 Cc. Doses.

B. Equal parts of pneumococcus cultures and toxic autolysates (undiluted or diluted 1 to 2).
C. Equal parts of pneumococcus cultures and broth.
D. Equal parts of toxic autolysates undiluted or diluted 1 to 2.

In every experiment in which pneumococcus cultures and toxic autolysates were injected together, controls of the same quantity of the pneumococcus culture alone (C) and the same quantity of the toxin preparation alone (D) were always injected separately into other guinea pigs.

Pneumococcus Strains Used.—The strains of Types I and II of the pneumococcus used in this work were the same as those used previously. Both strains when injected intraperitoneally were very virulent for rabbits, mice, or guinea pigs.

In these experiments we used only those filtrates which would kill guinea pigs within 24 hours, in undiluted doses of 0.2 cc. when injected intratracheally.

Technical Procedure.—18 to 24 hour broth cultures of Pneumococcus I and Pneumococcus II were used in this work. When there was profuse growth, the culture was diluted with broth to obtain the slight clouding desired. The pneumococcus culture was kept in ice water throughout the experiment.

Immediately before inoculation the tube containing the toxic filtrate was chilled, the vaseline seal opened, and the filtrate pipetted into a narrow chilled tube. This tube was kept in ice water throughout the experiment. Just before the injection, 0.2 cc. of the pneumococcus culture and 0.2 cc. of the toxin were pipetted into another iced tube, mixed, and taken up into an iced syringe; 0.2 cc. of this mixture was then immediately injected intratracheally into a guinea pig of 180 to 300 gm. A fresh mixture was always prepared before each injection, and the same precautions as to chilling, etc., were carried out with both control preparations of pneumococcus cultures and broth (C) and of toxic autolysate and broth (D).

(*B*) *Results From the Intratracheal Injection of Pneumococcus Culture and Toxic Autolysates.*

Forty-five guinea pigs were injected with mixtures of toxin and pneumococcus cultures. With one exception all died with pneumonic lesions and positive lung cultures. These forty-five guinea pigs may be conveniently grouped as follows:

1. 8 guinea pigs (18 per cent) which died in 6 hours or less.
2. 35 guinea pigs (77 per cent) which died in from 18 to 72 hours.
3. 2 guinea pigs (5 per cent) which survived longer than 3 days.

In this series are included a certain number of animals which were injected intraperitoneally 18 to 24 hours previous to the intratracheal injection of pneumococcus culture and autolysate with normal serum, either rabbit or horse, or with certain heterologous antipneumococcus horse sera. It will be shown in a later report that none of these sera had an appreciable effect upon the subsequent intoxication and infection caused by the intratracheal injections of mixtures of toxic autolysates and pneumococcus cultures.

1. Guinea Pigs Dying within 6 Hours after the Intratracheal Injection of Pneumococcus Culture and Toxic Autolysate.

These guinea pigs showed the same symptoms as those which had been injected with 0.2 cc. of a strong toxin alone. They were markedly dyspneic directly after the injection and continued in this condition

until death. At autopsy, the appearance of the lungs of these animals was also similar to that found in the guinea pigs which had been inoculated with toxin alone. Cultures from the lungs always gave a growth of the pneumococcus, and positive cultures were obtained from the heart blood in five of eight pigs. We believe that these animals were exceptionally susceptible to the toxin and probably would have died had 0.2 cc. of toxin diluted 1 to 2 been inoculated without pneumococcus culture.

Histological Findings.—Five animals (8-68, 7-59, 10-13, 43-74, and 7-07) of this group were studied histologically. In the two which died after 1¼ and 1½ hours respectively, there had already developed loose areas of consolidation about the large bronchi in the hilus region. In these pneumonic patches the alveoli contained an exudate with fairly abundant polymorphonuclears; red corpuscles were very numerous; there was a slight amount of delicate fibrin. About the larger vessels and bronchi the tissue was intensely edematous and infiltrated with wandering cells, including many polymorphonuclears. The lymphatic vessels were distended. In the unconsolidated portions of the lung the congestion was intense; there were hemorrhages and moderate alveolar edema. Pneumococci were not found at this stage.

The remaining three animals of this group, dying 2, 3, and 5½ hours after injection, showed virtually the same lesions, save that there was extension of the pneumonic areas, and an increased amount of fibrin. At this time, however, pneumococci were already present in considerable numbers, and in one, which died after 2 hours, an amazing multiplication of the bacteria had occurred.

2. Guinea Pigs Dying in from 18 to 72 Hours after the Intratracheal Injection of Pneumococcus Culture and Toxic Autolysates.

These animals did not appear sick or appeared only slightly sick after the injection. On the following day, however, they were all extremely ill, suffering from severe dyspnea which became progressively worse until death. At autopsy, from ⅓ to ⅔ of the total volume of both lungs was found enlarged and consolidated. The lower lobes were most frequently affected but consolidation was often present also in the middle and upper lobes. In all but one case both lungs were involved; in this instance, however, the entire right lung was

consolidated, and the left normal throughout. The pneumonic areas were deep red, yellowish gray, or greenish gray, the gray portions adjoining the hilum and the red portions surrounding them. The normal lung tissue was always at the margins of the lobes. The cut surface of the gray areas was yellowish gray and granular. In several of the guinea pigs which died in approximately 2 days, there was a cellulitis of the chest or abdominal walls. Cultures of the heart and lungs of this group always showed profuse growth of pneumococci.

Histological Findings.—Thirty-one guinea pigs of this group were studied. Although there were slight individual variations as regards the extent of the lesions, the pathological changes were on the whole surprisingly uniform. In almost all instances the greater part of both lungs was in a state of pneumonic consolidation. As was evident from the gross appearance, whenever a portion of the lobe remained unconsolidated, it was that at the margins, or distal from the hilic region.

In the earlier stages, the picture was in all essentials identical with that in the corresponding stages of lobar pneumonia in man. The alveoli were filled with an exudate composed predominantly of polymorphonuclear leucocytes; large mononuclears were not abundant, and when present could not readily be distinguished with the methods used from the exfoliated epithelium. The alveolar cells still attached to the septal wall were greatly swollen, and their cytoplasm vacuolated. Fibrin was irregularly present, but on the whole less abundantly and conspicuously than in human pneumonia. The capillaries in the earlier stages were engorged with well preserved erythrocytes. With the progress of the lesion, and the passage of the red blood cells into the alveoli, they frequently appeared collapsed and empty. Their basement membrane often seemed swollen and thickened. In the later stages, the red cells—those within the capillaries as well as those forming part of the alveolar exudate—appeared to fuse into agglomerated masses staining intensely with eosin, and such fused masses of erythrocytes formed capillary thrombi and entered into the matrix of the alveolar exudate. In the latter stages of the lesions, the derivation of this pink-staining material in which were embedded the fragmented leucocytes, was not apparent, but by following successive phases, it seemed clear that the hemoglobin

masses from the fusion of red blood corpuscles were the source of this material. The usual picture of hemolysis, with the preservation of the shadows of the hemolyzed cells, was never seen; nor was the picture that of rhexis.

The leucocytes of the exudate in the earlier stages were swollen and hydropic—often with a distinct cell membrane and a watery clear cytoplasm. Later the nucleus underwent fragmentation. The fibrin, delicate at first, later became swollen and the threads merged into larger clumps and masses.

The most striking and interesting feature of these experimental lesions, however, was the unrestrained growth of the pneumococci. In all the animals of this group, enormous numbers of diplococci were present, in many alveoli forming dense colonial masses. In sections stained by the Gram-Weigert method, the bacteria were so numerous as to impart a blue color to the section. They were often present in abundance in the edematous tissue about the blood vessels and the bronchi, as well as in the alveolar exudate.

3. Guinea Pigs Which Survived 7 and 14 Days Respectively after the Intratracheal Injection of Pneumococcus Culture and Toxic Autolysates.

The guinea pig which died in 7 days was slightly dyspneic after the first day and continued in this condition until death. At autopsy, the lungs were collapsed but appeared otherwise normal except for the left lower lobe which was slightly consolidated and of a translucent grayish color. Cultures from the heart and left lower lobe of this guinea pig gave a profuse growth of pneumococci. Histologically, the section through the left lower lobe showed the capillaries to contain little blood; the endothelial nuclei were swollen and hyperchromatic, the alveolar epithelial cells large and vacuolated. In the connective tissue about the main bronchus were several circumscribed masses of polymorphonuclear cells—the only evidence of an acute inflammatory reaction. The edematous peribronchial tissue was becoming organized by the ingrowth of fibroblasts. The lesions indicated recovery from a previous pneumonia.

Guinea Pig 84 died on the 14th day, having shown no signs of illness at any time. No gross or microscopic lesions were found to indicate

the occurrence of a previous pneumonia. Either the animal was exceptionally resistant to the autolysate and pneumococci or there had been a technical error in the inoculation.

(*C*) *Results from the Intratracheal Injection of Pneumococcus Cultures and Broth.*

Ten guinea pigs were injected with mixtures of pneumococcus cultures and broth. None of these became dyspneic at any time; only three of them died, two on the 5th day and one on the 7th. At autopsy, the gross appearance of the lungs of these three was normal. Cultures from the heart blood yielded a profuse growth of pneumococci.

Histological Findings.—Ten guinea pigs were studied histologically, the three which died, described above, and seven additional guinea pigs which were killed at intervals of 1 to 4 days after the inoculation of varying amounts of culture. Lesions were found only in the animals which were killed during the first 48 hours. They consisted of a sparse emigration of polymorphonuclear leucocytes into a few alveoli only, accompanied by occasional red blood corpuscles. There was no fibrinous exudation. Indeed there was no massive consolidation comparable to that regularly occurring in the animals of Group B already described. Pneumococci were found in the sections of but two of this series—6-28, killed after 27 hours and 6-39, killed after 2 days—and not in excessive numbers. Both of these animals received a very much larger dose of the microorganism than was given to the animals of Group B, which received toxic autolysates in addition to the living pneumococci.

The three guinea pigs dying on the 5th and 7th days showed no pulmonary lesions whatsoever in spite of the general septicemia.

(*D*) *Results from the Intratracheal Iniections of Toxin and Broth.*

Ten guinea pigs were injected with toxin and broth. Of these, three pigs died; two died 2 hours after the injection, and one died 48 hours after the injection with characteristic symptoms and pathology of a guinea pig injected with 0.2 cc. of a strong toxin, described above, *viz.*, in the two pigs which died in 2 hours the lungs were patchy deep red and pink throughout; in the pig which died in 48 hours $\frac{2}{3}$ of the

lungs was consolidated and deep red. The cultures of the lungs and heart of these three pigs were sterile. The other seven guinea pigs in this series were more or less dyspneic after the inoculation but had practically recovered from this sickness by the next day and survived.

DISCUSSION.

If we contrast the symptoms and pathology in these three groups, it is evident that there are striking and significant differences in the reaction of the animal. In agreement with previous workers it has been impossible to produce extensive pneumonic lesions by the injection of living pneumococci alone, even in large doses. The lesions which followed the intratracheal administration of the living organism appeared early and were mild and transitory in character even when the doses were greatly in excess of those used in the other groups. The animals which succumbed died of septicemia without lung involvement.

In striking contrast to this behavior was the reaction of the animals which received in addition to the living pneumococci, a small dose of the toxic autolysate. A certain proportion of these died within a few hours with shock-like symptoms; and from our observation upon the effect of toxic autolysate alone it would seem that the poisonous effect of this substance was primarily responsible since death occurred before there had been opportunity for the multiplication of the bacteria. The changes in the lung may be summarized as an intense hemorrhagic edema with very early inflammatory reaction.

In animals surviving this first shock there developed regularly massive areas of consolidation often of the lobar type and accompanied by an amazing multiplication of the pneumococci throughout the lung. The growth of the bacteria seemed indeed to outstrip the inflammatory reaction, since one found them even in areas outside of the pneumonic zones. It is clear that the toxic substances have in some way blocked the defensive mechanism and made conditions favorable for unrestrained growth. We shall not at this time attempt to analyze this further, or speculate as to the factors responsible for this striking phenomenon.

Experiments also demonstrated the marked toxicity of the bacterial autolysates prepared after the described manner. The lesions pro-

duced were intense and widespread in the lungs. The chief differences noted histologically between the reactions to the toxic autolysates alone, and the reaction when the poisonous substance was accompanied by the introduction of the living organism, are that there is less fibrin in the former group and the exudate appears to be somewhat looser in character. The alteration of the red blood corpuscles which was a striking feature in the presence of the living bacteria, was not seen with the toxic autolysate alone. Furthermore, as one might expect, there is an attempt at recovery and repair on the part of the animals which have been allowed to live for 3 days or more. These observations upon the primary toxicity of the autolysates brings up, of course, the interesting problem as to the rôle of similar substances formed *in vivo*, in the production of pneumonia in man.

CONCLUSIONS.

1. Anaerobic autolysates of pneumococci, prepared according to the method described, are highly toxic for guinea pigs when injected intratracheally in dosage of 0.2 cc. Death occurs either within a few hours (36 per cent) or within 3 days. In the early deaths there is intense hemorrhagic edema of the lungs with beginning inflammatory reaction; in animals surviving for 18 hours or longer extensive areas of pneumonia are produced.

2. The intratracheal injection of virulent living pneumococci is followed by transient slight lesion with recovery, or by later death from septicemia without pneumonic lesions.

3. The addition of a sublethal dose of toxic autolysate to living pneumococci alters the reaction of the animal, so that there develops extensive pneumonia associated with unrestrained multiplication of the organism.

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EXPLANATION OF PLATES.

PLATE 18.

FIG. 1. G. P. 8-49. Killed 29 hours after intratracheal injection of toxic autolysate alone. Patchy areas of pneumonia and diffuse edema.

FIG. 2. G. P. 8-53. Killed 24 hours after intratracheal injection of pneumococci alone. Sparse emigration of polymorphonuclears into a few alveoli. No pneumonic consolidation.

PLATE 19.

FIG. 3. G. P. 10-29. Killed 48 hours after intratracheal injection of pneumococci plus toxic autolysate. Diffuse pneumonic consolidation, with abundant growth of pneumococci.

FIG. 4. G. P. 6-27. Died 24 hours after intratracheal injection of toxic autolysate and pneumococci. Unrestrained growth of pneumococci in lung. (Gram-Weigert stain.)

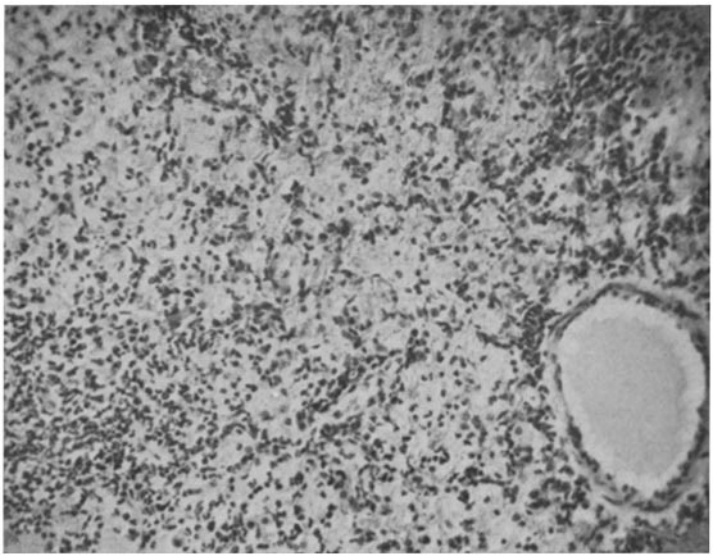

FIG. 1.

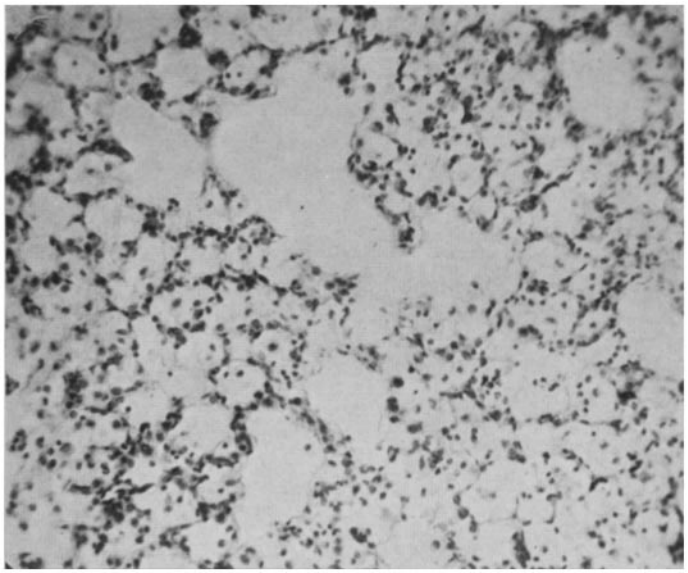

FIG. 2.

(Parker and Pappenheimer: Pneumonia in guinea pigs. I.)

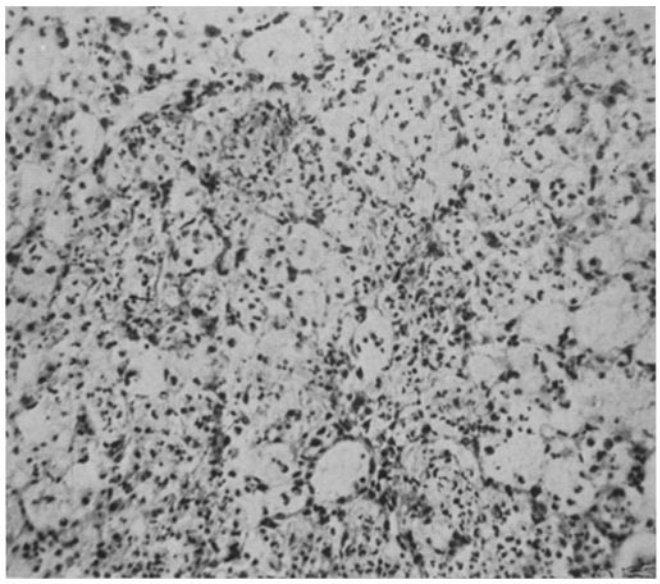

FIG. 3.

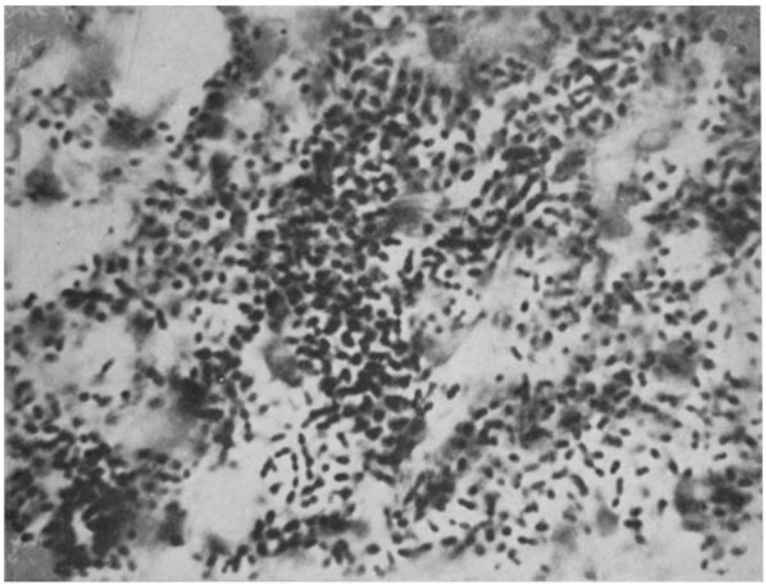

FIG. 4.

(Parker and Pappenheimer: Pneumonia in guinea pigs. I.)