INTERSTITIAL BRONCHOPNEUMONIA

I. Similarity of a Toxin Pneumonia to That Produced by the Viruses

By DOUGLAS H. SPRUNT, M.D., DONALD S. MARTIN, M.D., and JARRETT E. WILLIAMS, M.D.

(From the Departments of Pathology and Bacteriology of the Duke University School of Medicine, Durham)

Plates 5 to 7

(Received for publication, April 3, 1935)

In 1861, Bartels (1) described the presence of a number of cells, many of which were apparently monocytes, in the interstitial tissue of the lungs of patients dying of pneumonia complicating measles. Delafield (2), in 1884, stated that this type of interstitial reaction was quite common in pneumonias. Interest in this type of pneumonia was renewed during the world war by MacCallum (3) and Opie and his associates (4). The cases reported by MacCallum followed measles and those of Opie complicated influenza. MacCallum (3) interpreted the interstitial mononuclear reaction as evidence of a partial immunity of the host to the bacteria causing the pneumonia. He also gave it the name interstitial bronchopneumonia.[1]

Muckenfuss and his associates (5), in 1929, showed that small amounts of vaccine virus injected into the trachea of rabbits caused an interstitial proliferative mononuclear reaction in the lungs, while larger amounts produced an edematous, hemorrhagic consolidation with irregular areas of necrosis. The former type of reaction had been previously noted in psittacosis by Rivers and Berry (6) and others. Later McCordock and Muckenfuss (7) produced lesions similar to those observed in an interstitial bronchopneumonia by following the

[1] Interstitial bronchopneumonia is a thickening of the interstitial tissue of the lungs. This thickening is particularly noted around the bronchi and is due to an increase in the number of mononuclear cells present. In addition to this change in the interstitial tissue there is also involvement of the bronchioles and surrounding alveoli with the usual polymorphonuclear response. Many of the alveoli, however, are filled with either large mononuclear cells or fibrin.

injections of virus with intratracheal inoculations of bacteria. From a study of a number of pneumonic lesions and from reports in the literature, they concluded that interstitial bronchopneumonia occurred after measles, pertussis, and epidemic influenza. Measles is a recognized virus disease. They averred, furthermore, that pertussis and influenza are probably virus diseases on the grounds of a similarity of the anatomical lesions associated with them to those produced by intratracheal injections of vaccine virus. The finding of inclusion bodies in pertussis by McCordock (8) and Rich (9) was given as additional proof. Hence they regarded interstitial bronchopneumonia as the typical response of the lungs to the combined action of a virus and a bacterium, although in no sense specific for a particular virus or bacterium.

While no one doubts that the combination of virus and bacterium will cause such an interstitial reaction, there is some question whether this is the only agent or agents which will produce such a reaction. Olcott (10) reports three cases of acute Friedländer's bacillus pneumonia in which the mononuclear cells predominated. Blake (11) states that extreme care should be used in attempts to relate mononuclear cells to virus or bacterial infections. Foot (12) says that mononuclear cells are much more frequent in pneumonia than is generally thought. In examining the lungs from a number of cases in which there was no known virus infection, we have frequently found sufficient mononuclear and interstitial reaction to make the diagnosis of interstitial bronchopneumonia. Of course it is possible that there was an unrecognized virus infection, but it seems more likely that the reaction was dependent on some bacterial products. As toxins react directly on cells and display certain activities similar to those of viruses (high grade immunity following infection, appearance of specific neutralizing substances in the blood of an infected host, and potency as an antigen), it was thought that a study of the reaction produced in lungs by toxins would aid in understanding the nature of this type of pneumonia. With this idea in mind the following experiments were undertaken.

Methods and Materials

The animals used in the experiments were young adult rabbits. Only rabbits which were not carriers of *Bacterium lepisepticum* and *Bacillus bronchisepticus*

were used. The presence or absence of these organisms in the nares was determined by means of cultures after silver nitrate instillations, as suggested by Meyer (13).

Toxins.—Staphylococcus toxin was made from the strain of *Staphylococcus aureus* employed by Rigdon and his associates (14), and was prepared by the method described by Parker, Hopkins, and Gunther (15).

The streptococcus toxin was prepared from a strain of *Streptococcus hemolyticus* which was isolated from a case of erysipelas. The toxin was made by filtering through a Seitz filter a culture of the organism grown for 7 days in beef infusion broth containing 0.5 per cent blood and 0.5 per cent dextrose.

The diphtheria toxin was prepared by diluting with normal saline the dried toxin obtained from the North Carolina State Laboratory of Hygiene.

Inoculation.—The animals were lightly anesthetized with ether. The hair over the neck region was clipped and then iodine poured over the denuded skin. A small incision was made in the midline just below the larynx. The trachea was exposed and the material, which was contained in a tuberculin syringe fitted with a 27 gauge needle, was injected directly into its lumen. The head of the animal was then elevated and the incision was closed with one suture.

Dose.—In every instance the total volume of material injected was 1.0 cc. Normal saline was always the diluent except in a few of the earlier experiments where beef extract broth was used. It was found in preliminary experiments that doses of from 0.1 to 0.3 cc. of the staphylococcus toxin produced a proliferative type of lesion, while larger doses produced a hemorrhagic and necrotizing lesion.

Necropsy.—Most of the animals were killed 72 hours after injections of the *Staphylococcus aureus* toxin, as the lesions were found at this time to be most actively proliferative. A number of animals injected with the *Staphylococcus. aureus* toxin were killed at intervals of from 3 hours to 2 weeks after inoculation. No symptoms were noted in the animals during the progress of the infection except in the case of two half grown rabbits that died shortly after the injections were made. These rabbits were slightly dyspneic.

Animals were killed by a blow at the base of the skull and the necropsies were performed at once. The pleural cavity was opened aseptically, and a portion of the lung at the point of maximum involvement was cut out with sterile scissors. This portion was divided into three parts. One part was streaked on a blood agar plate, and each of the two other pieces was placed in a tube of beef infusion broth. One tube was sealed with vaseline for anaerobic growth. The plate and broth tubes were incubated for 7 days before being reported as sterile.

Fixation and Stains.—After the lungs were removed from the body they were sectioned and fixed in Zenker's (Helly modification) solution and 10 per cent formalin. The sections were embedded in paraffin and stained with hematoxylin and eosin and for bacteria according to MacCallum's method. The lungs of 8 rabbits were not treated as described above, but for the sake of better histological preparations were removed without culturing, inflated with air through the bron-

chi, and then fixed *in toto* by injection of Zenker's fluid through the pulmonary artery. After fixation the lungs were sectioned and stained with hematoxylin and eosin and for bacteria as described above.

EXPERIMENTAL

Controls.—In order to be certain that the reactions to be described in the lungs were due to the toxin used and not to extraneous substances which may have been in the injected material or to a dormant virus, the following control experiments were conducted. In all cases the animals were killed 72 hours after inoculation.

Heated Toxin.—3 rabbits were inoculated with 0.3 cc. of the *Staphylococcus aureus* toxin which had been heated to 60°C. for 1 hour. A few focal areas showing slight proliferative changes were found.

Toxoid.—2 rabbits were injected intratracheally with 0.3 cc. of a staphylococcus toxoid obtained from the Lederle Laboratories. A few small areas showing a slight increase in mononuclear cells were seen.

Staphylococcus albus.—4 rabbits were injected with material prepared exactly as was the *Staphylococcus aureus* toxin except that a *Staphylococcus albus* was used. This material produced slight lesions when 1.0 cc. of the undiluted material was injected. Doses smaller than this resulted only in the accumulation of small numbers of polymorphonuclear cells in the first 48 hours.

Toxin-Antitoxin Mixtures.—5 rabbits were injected with a mixture of the *Staphylococcus aureus* toxin and varying amounts of Lederle staphylococcus antitoxin. The mixture was allowed to stand at room temperature for 1 hour before injection. No reaction was observed in the lungs of 4 which received more than 0.1 cc. of antitoxin for 0.4 cc. of the toxin. In one animal which received 0.1 cc. of the antitoxin and 0.9 cc. of toxin a slight proliferation of the lining cells of the alveoli was noticed.

Actively Immunized Animals.—2 rabbits were immunized by subcutaneous injections of *Staphylococcus aureus* toxin, starting with 0.1 cc. and increasing the dose until they were receiving injections of 2 cc. 4 weeks after the last immunizing dose they were injected intratracheally with 1 cc. of the toxin. The immunizing injections reduced the amount of reaction in the lungs but did not entirely prevent it. Protection tests conducted in mice showed that 0.05 cc. of the rabbit serum protected mice against a lethal dose of the toxin.

Passively Immunized Animals.—6 rabbits received Lederle staphylococcus antitoxin intravenously 24 hours before the intratracheal injections of toxin were made. Doses of serum of from 1 to 8 cc. failed to prevent the development of some mononuclear response, although the reaction was much less than in the non-immunized animals.

Virus Control.—A rabbit lung with a typical lesion was triturated with an abrasive. A 10 per cent suspension was made and 1 cc. of this was injected into

each of 3 rabbits. The lungs of these rabbits showed some red blood cells in the alveoli but no mononuclear or proliferative reaction.

Experiments.—As it has been shown by Muckenfuss and his associates (5) that small amounts of vaccine virus injected into the lungs of rabbits cause a proliferative and mononuclear reaction, while larger amounts produce an edematous, hemorrhagic consolidation with irregular areas of necrosis, the following experiments were designed to study the effects of both small and large amounts of a toxin. The staphylococcus toxin was chosen for the bulk of the experiments, inasmuch as it was found to produce satisfactory lesions consistently when used in small amounts. The results were checked in later experiments with other kinds of toxins. As the only significant changes were found in the lungs, data concerning the other organs will not be given. Experiment 1 was designed to show the effect of the small amounts of toxin, while Experiment 2 deals with larger amounts of the same agent. As 0.3 cc. of the toxin produced proliferative lesions in some rabbits and necrotizing lesions in others, the rabbits receiving this dose were arbitrarily placed in either Experiment 1 or 2 on the basis of the lesion produced.

Experiment 1.—24 rabbits were used in the experiment, and each received either 0.15 or 0.3 cc. of the staphylococcus toxin. 12 rabbits were killed 3, 6, 12, 24, and 48 hours and 7 and 14 days respectively after injection, and 12 rabbits were killed at 72 hours, a time at which the proliferative reaction had been found to be at its height.

Morbid Anatomy. Gross.—No appreciable change is seen until 12 hours have elapsed since inoculation, at which time the capillaries in part of the lungs are engorged, but little or no edema is present. At the end of 48 hours scattered areas of consolidation are evident. These areas are purple and their surface on section is moist. The lungs at the end of 7 days exhibit firm grey areas which on section are grey, dry, and translucent. After 14 days there is no further change in the appearance of the lungs. Throughout the experiment the pleura remains smooth and glistening.

Microscopic.—Animals killed 3 hours after inoculation have a polymorphonuclear cell reaction which is present throughout the interstitial tissue of the lung and is most intense around the bronchioles and blood vessels. There is, however, no phlebitis or arteritis. A few alveoli contain polymorphonuclear cells and an occasional mononuclear cell. There is a slight amount of edema present in the walls of the bronchioles and in the adventitia of the blood vessels. Within 24 hours after the inoculation many alveoli, as well as the interstitial tissue, contain a large number of polymorphonuclear cells. There is also considerable edema.

After 48 hours the number of polymorphonuclear cells and the amount of edema have increased. At this time, however, one sees the first evidence of another reaction; namely, thickening of the alveolar walls. The cells causing the thickening are round or cuboidal with vesicular nuclei and scanty, poorly stained cytoplasm. At this time no attempt will be made to state the nature of these cells or to say whether they have the ability to change into phagocytic cells. They are generally seen lining the alveoli but may also be observed as solid masses or as a syncytium of cells. In addition there are a few cells with many nuclei which resemble foreign body giant cells, and a number of monocytes and macrophages (Fig. 3).

At 72 hours the alveolar walls are markedly thickened, and in places on superficial examination there appears to be a large number of bronchioles (Figs. 1 and 2). On closer inspection these are seen to be alveoli which are lined with large cells having vesicular nuclei, small nucleoli, and scanty, poorly stained cytoplasm. Many of the nuclei are in various stages of mitosis (Figs. 5 and 6). Mixed with these cells are degenerating polymorphonuclear cells, debris, and red blood cells. Extensive hemorrhage is present in other alveoli. The perivascular lymphatics are filled with a large number of lymphocytes and some mononuclear cells (Fig. 4).

At the end of 7 days one sees areas in the lungs in which the lumens of the alveoli are obliterated by the proliferation of cells (Fig. 7). In other areas, however, alveoli containing and lined with large cells are still found. These cells have round or slightly oval vesicular nuclei and scanty cytoplasm and are thought to be proliferating epithelial lining cells. A few mitotic figures are seen. Besides these elements an occasional polymorphonuclear cell and a small number of mononuclear cells with eosin-staining granules in the cytoplasm are found. The granules are about the size of the granules in rabbit polymorphonuclear cells.

The 14 day rabbit lungs resemble in every way the 7 day ones with the exception that fewer cells are seen.

Although no studies were made later than 14 days after inoculation, it is thought, since no fibrous tissue was seen, that the lungs would return to normal if sufficient time were allowed.

Experiment 2.—8 rabbits were used, each of which received intratracheally from 0.3 to 0.5 cc. of toxin. In addition to these animals, each of 3 baby rabbits received 0.1 cc. of toxin. Some of the rabbits died within 18 hours, but the majority were killed at the end of 72 hours.

Morbid Anatomy. Gross.—All the animals regardless of the time of death have boggy consolidated lungs. The pleural cavities contain slightly more fluid than usual. Numerous purple areas of consolidation are seen. The cut surfaces of the lungs are covered with a large amount of bloody fluid. Some infarct-like areas of necrosis are seen. When the areas extend to the pleura, it is roughened; but in uninvolved areas it is smooth and glistening.

Microscopic.—The main difference between lesions observed in this experiment and those described in Experiment 1 is that here, in the beginning, the epithelial

tissue and the underlying structures are actually killed and no proliferation takes place (Fig. 8). These necrotic areas are infiltrated with a large number of polymorphonuclear leukocytes, and healing of them results in the formation of definite fibrous tissue.

In order to ascertain whether the results of the above experiment with staphylococcus toxin could be obtained with other toxins the following experiment was performed.

Experiment 3.—11 rabbits were used in this experiment. All of the animals were killed 72 hours after the injection of the toxin. 25, 50, and 100 skin test doses of the diphtheria toxin were given to 5 rabbits and 0.3 and 0.5 cc. of the streptococcus toxin were employed in 6 rabbits.

Morbid Anatomy.—The lungs from these rabbits resembled in every detail those described in Experiment 1.

Summary of Experiments

The injection of relatively small amounts of toxin into the trachea of rabbits resulted in a reaction in the tissue evidenced by an exudation of large numbers of polymorphonuclear cells and some edema. The cells were situated mainly in the interstitial tissue and particularly around the bronchi. Some, however, were present in the alveoli. This type of reaction was observed as late as 48 hours after inoculation, at which time the polymorphonuclear elements began to decrease in number. The walls of the alveoli and the tissues surrounding the bronchi and bronchioles then became infiltrated with lymphocytes and monocytes. In addition to the presence of these cells, the walls of the alveoli were definitely thickened with cuboidal cells which had vesicular nuclei and scanty, pale staining cytoplasm. It was impossible to determine the exact nature of these cells, but it is our impression that they were epithelial cells. Mitotic figures were seen in many of the cells present. The reaction observed 2 weeks after injection of the toxin and after the debris of the early polymorphonuclear reaction had been cleared away was mainly one of proliferation of the cells of the alveolar walls. It is thought that complete resolution would occur as no fibrous tissue was seen.

When large amounts of toxin were injected, the lesions became hemorrhagic and necrotic with polymorphonuclear cells infiltrating the necrotic areas.

In both types, particularly in the hemorrhagic form, some poly-

morphonuclear involvement of the arteries with, in certain instances, thrombus formation was found. The necrotic areas resemble infarcts, but it is thought that the necrosis occurred simultaneously with the vascular changes and not subsequent to them. This type of lesion never went on to complete resolution but always left scar tissue in the place of the necrosis.

DISCUSSION

Before we can say that the pneumonia described above is the result of the injected toxin, the possibility that the lesions were due to any of the following three causes should be excluded: First, that they were produced by bacteria. The sterility of the aerobic and anaerobic cultures of the lungs and the absence of bacteria in the microscopic preparations adequately refute this possibility. Second, that the toxin acted as a stimulus to an unrecognized virus lying dormant in the tissues. This possibility is excluded by the absence of inclusion bodies in the microscopic preparations, together with the failure to pass the disease to other rabbits. Third, that the lesions were non-specific. This assumption may be refuted by the absence of lesions when toxin-antitoxin mixtures were used and the marked reduction of the extent of the lesions when toxoid or heated toxin was used. Additional evidence that the lesions were not non-specific is the absence of lesions following the injection of a material similar to the *Staphylococcus aureus* toxin except that a *Staphylococcus albus* was employed in its preparation. Furthermore, although active immunization of rabbits by multiple subcutaneous injections of *Staphylococcus aureus* toxin failed to produce complete protection of the lungs, such treatments did decrease appreciably the intensity of the reaction. Essentially similar results were obtained in the animals that received large injections of the antitoxin intravenously the day before the intratracheal inoculation of the toxin was made. In the latter experiment, however, the intensity of the lesions bore an inverse ratio to the amount of antitoxin injected. In the light of the above experiments it is obvious that small doses of *Staphylococcus aureus* toxin and, to a lesser degree, diphtheria and streptococcus toxins, are capable of producing a mononuclear and proliferative pneumonia; whereas large doses of *Staphylococcus aureus* toxin cause an edematous, hemorrhagic,

and necrotizing pneumonia. This reaction is similar to that obtained by Muckenfuss and his associates (5) with vaccine virus. The only difference in the two sets of experiments is that in ours a large number of polymorphonuclear cells were present in the first 48 hours, whereas in the vaccine virus experiments only a few were seen. It is not unlikely that these cells appeared in response to some impurity in the preparation used and not to the toxin itself, inasmuch as the same amount of polymorphonuclear response was obtained with *Staphylococcus albus* preparations. It is thought that this type of response was due to the presence either of disintegrated bacteria or of substances in the culture medium.

The mononuclear proliferative pneumonia described by us is also similar to psittacosis pneumonia as seen in man and monkey and to the pneumonia complicating pertussis.

In view of these experiments we feel justified in maintaining that the proliferative and mononuclear portion of interstitial bronchopneumonia may be caused by bacterial toxins as well as by viruses.

We wish to call attention to the similarity of the pneumonias produced by toxins and viruses to that obtained by Winternitz and his associates (16) with acids and gases. Their results differed from ours in certain respects; *i.e.*, the epithelium of the bronchi and bronchioles was stimulated to a greater extent than in our experiments. Such facts suggest the possibility that this type of response of the lungs will result from any form of injurious agent that damages the cells without killing them. The injurious agent acts as a stimulus to cell growth and brings about a proliferation of the cells. However, if the amount of the agent is sufficient to cause the death of the tissue, a polymorphonuclear response is elicited by the dead tissue. In current experiments to be reported at a later date, this point and others dealing with the effect of combinations of bacteria with toxins are being investigated.

CONCLUSIONS

Bacterial toxins can produce a pneumonia similar to that caused by viruses, and the presence of a toxin-producing bacterium in the lungs may account for some of the interstitial bronchopneumonias observed in diseases other than those caused by viruses.

BIBLIOGRAPHY

1. Bartels, *Virchows Arch. path. Anat.*, 1861, **21**, 65.
2. Delafield, F., *Boston Med. and Surg. J.*, 1884, **111**, 484.
3. MacCallum, W. G., The pathology of pneumonia in the United States Army camps during the winter of 1917–18. Monograph of The Rockefeller Institute for Medical Research, No. 10, New York, 1919.
4. Opie, E. L., Blake, F. G., Small, J. C., and Rivers, T. M., Epidemic respiratory disease, St. Louis, C. V. Mosby Co., 1921.
5. Muckenfuss, R. S., McCordock, H. A., and Harter, J. S., *Am. J. Path.*, 1932, **8**, 63.
6. Rivers, T. M., and Berry, G. P., *J. Exp. Med.*, 1931, **54**, 129.
7. McCordock, H. A., and Muckenfuss, R. S., *Am. J. Path.*, 1932, **8**, 63.
8. McCordock, H. A., *Proc. Soc. Exp. Biol. and Med.*, 1931–32, **29**, 1288.
9. Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 346.
10. Olcott, C. T., (abstract), *Am. J. Path.*, 1933, **9**, 959.
11. Blake, F. G., (discussion of above paper by Olcott), *Am. J. Path.*, 1933, **9**, 959.
12. Foot, N. C., (discussion of above paper by Olcott), *Am. J. Path.*, 1933, **9**, 959.
13. Meyer, K. F., in Jordan, E. O., and Falk, L. S., The newer knowledge of bacteriology and immunology, Chicago, The University of Chicago Press, 1928, 607.
14. Rigdon, R. H., Joyner, A. L., and Ricketts, E. T., *Am. J. Path.*, 1934, **10**, 425.
15. Parker, J. T., Hopkins, J. G., and Gunther, A., *Proc. Soc. Exp. Biol. and Med.*, 1925–26, **23**, 344.
16. Winternitz, M. C., Smith, G. H., and McNamara, F. P., *J. Exp. Med.*, 1920, **32**, 205.

EXPLANATION OF PLATES

PLATE 5

FIG. 1. Section of rabbit lung 72 hours after intratracheal inoculation of *Staphylococcus aureus* toxin. A zone of infiltration surrounds the bronchus, and the alveolar walls are thickened. The lumina of the bronchi are clear. × 165.

FIG. 2. Same as Fig. 1 but showing a more intense reaction. The alveoli are lined with cells resembling the lining of the bronchi. × 165.

FIG. 3. High power of Fig. 1 showing types of cells found in the alveoli. Note absence of polymorphonuclear cells. × 730.

PLATE 6

FIG. 4. Section of rabbit lung 72 hours after intratracheal inoculation of *Staphylococcus aureus* toxin, showing marked mononuclear periarteritis. × 250.

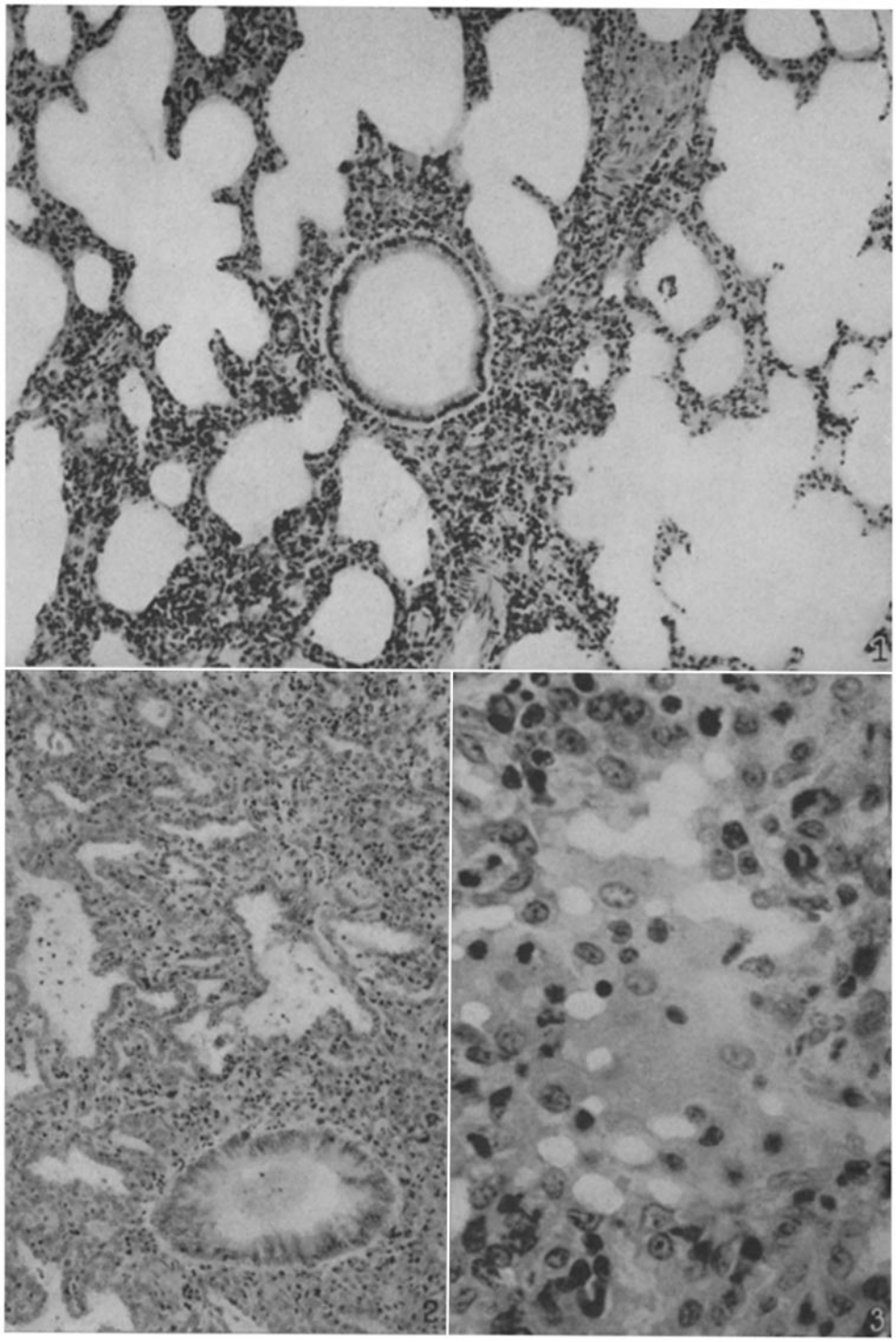
FIG. 5. Same as Fig. 4 but showing marked proliferation of cells in alveolar walls. × 250.

FIG. 6. Higher power of Fig. 14 showing mitotic figures. × 730.

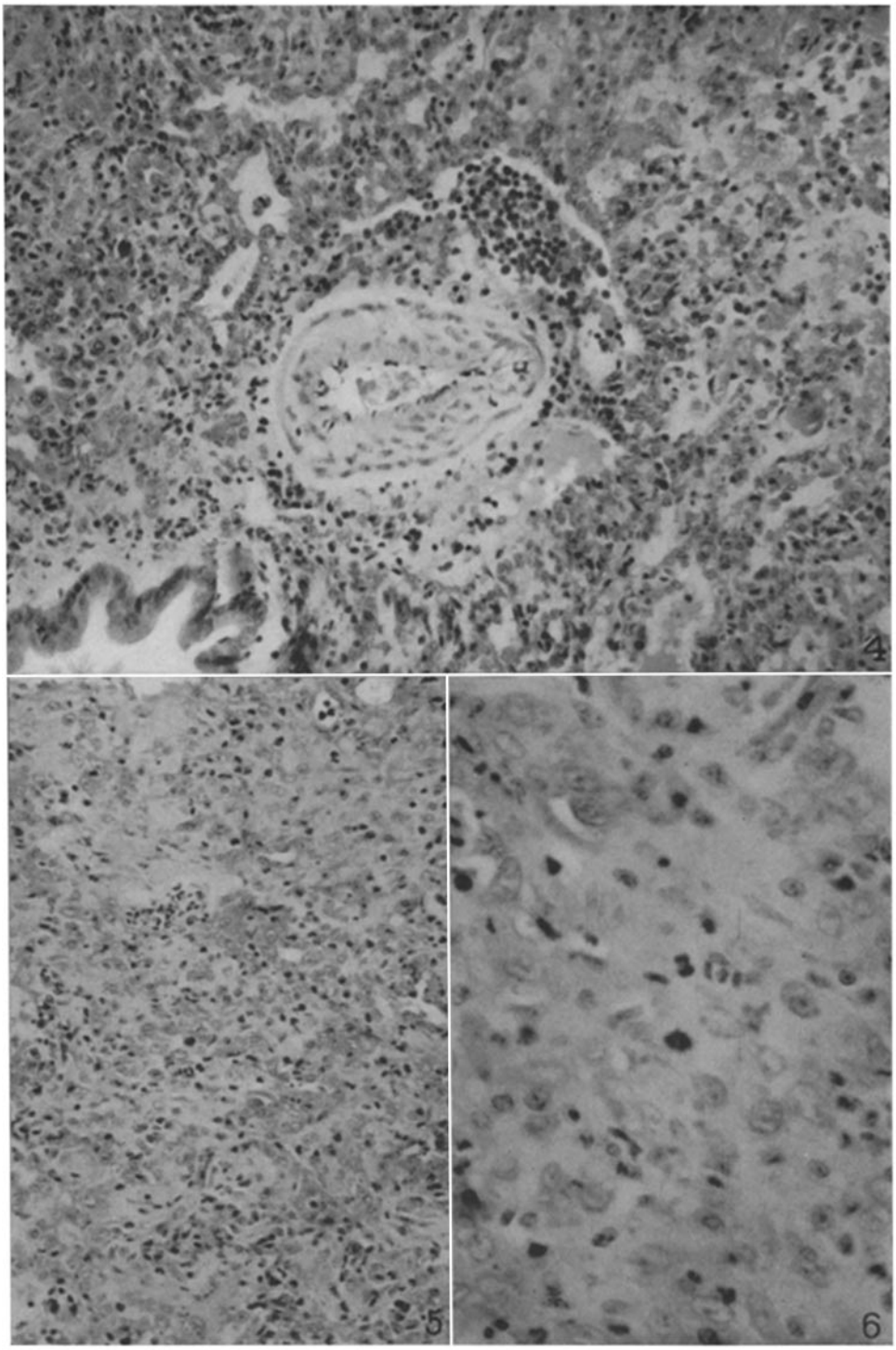
PLATE 7

FIG. 7. Section of rabbit lung 14 days after intratracheal inoculation of *Staphylococcus aureus* toxin. Note the syncytial-like mass of cells. × 165.

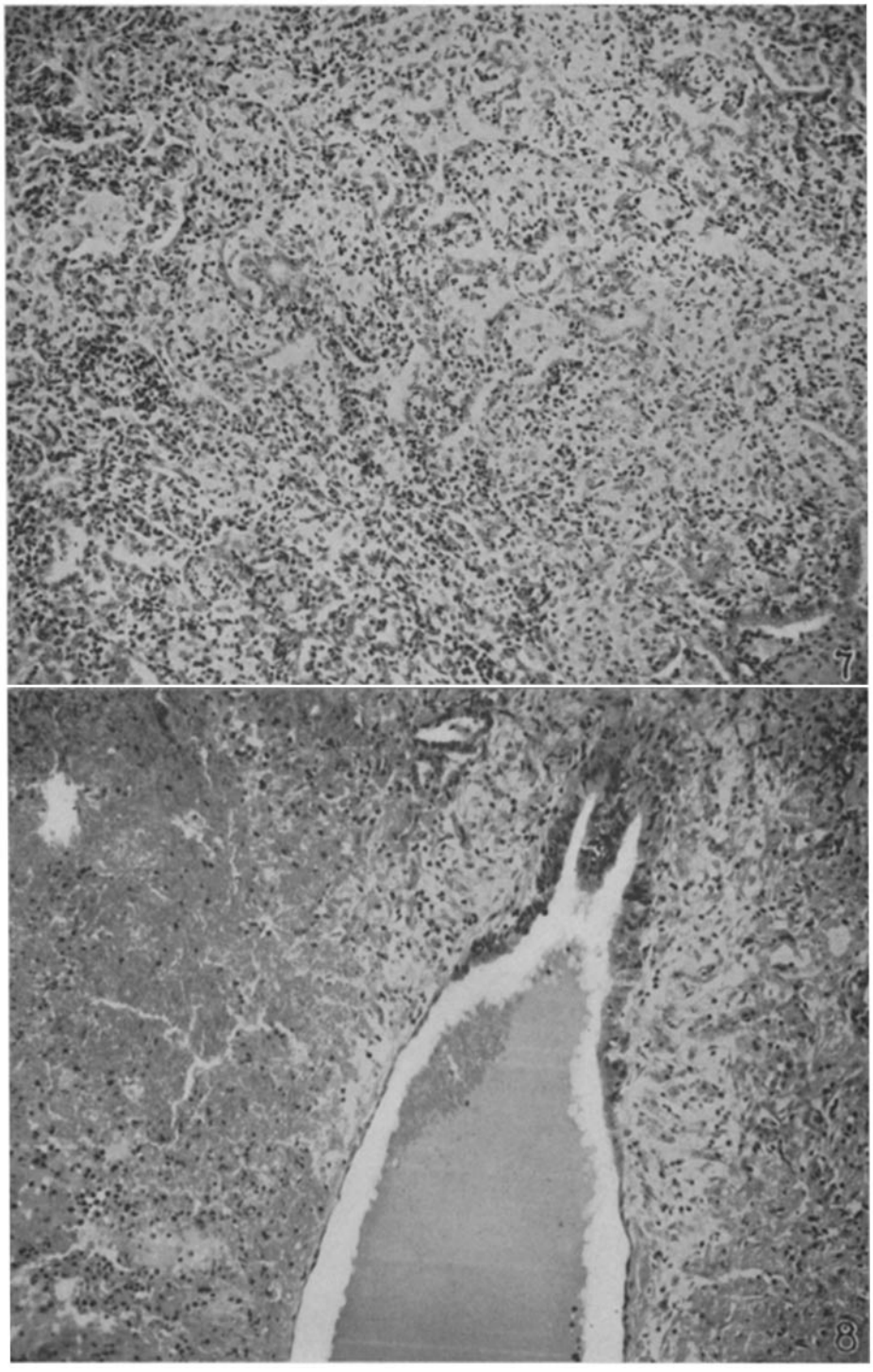
FIG. 8. Section of rabbit lung 72 hours after intratracheal injection of *Staphylococcus aureus* toxin. Note necrosis on the left. The bronchus is denuded of epithelium except in one place where there is proliferation. × 98.



(Sprunt *et al.*: Interstitial bronchopneumonia. I)



(Sprunt *et al.*: Interstitial bronchopneumonia. I)



(Sprunt *et al.*: Interstitial bronchopneumonia. I)