

STUDIES ON THE MECHANISM OF RECOVERY IN PNEUMONIA DUE TO FRIEDLÄNDER'S BACILLUS

II. THE EFFECT OF SULFONAMIDE CHEMOTHERAPY UPON THE PULMONARY LESION OF EXPERIMENTAL FRIEDLÄNDER'S BACILLUS PNEUMONIA*

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Both clinical and experimental studies indicate that infections due to Friedländer's bacillus may be favorably affected by sulfonamide chemotherapy (1-12). Little is known, however, concerning the mechanism of recovery from such infections in either human patients or laboratory animals. In the preceding paper the pathogenesis of experimental Friedländer's pneumonia in rats was described (13). The disease produced resembled acute Friedländer's pneumonia in man. The present report deals with the effect of sulfonamide chemotherapy upon the pulmonary lesion of this experimental infection.

Methods

The detailed techniques employed in the production of experimental Friedländer's bacillus pneumonia in white rats have already been described (13). The pneumonia produced was almost uniformly fatal in untreated animals. Blood cultures were taken from the tail at frequent intervals during the course of the infection, and all lungs were fixed in Zenker-formol solution according to the method of Loosli (14). Tissue sections were stained by the Gram-Weigert technique (15).

Sulfonamide Treatment.—Either powdered sulfadiazine or powdered sulfamerazine¹ was used in all experiments. Five gm. of the powdered drug was added to 100 ml. of a 10 per cent gum acacia mixture, and appropriate amounts of the resulting suspension were introduced into the stomach through a blunt cannula by way of the mouth. Three hundred mg. of sulfadiazine (6 ml.) was given as an initial dose, at the end of 12 hours and every 24 hours thereafter. When sulfamerazine was used, 24 hour maintenance doses of 200 mg. (4 ml.) were found to be sufficient to maintain an adequate blood level. The concentrations of drugs maintained in the blood by the two dosage schedules in both normal and infected animals are charted in Text-fig. 1.

Tests for Antibody.—The methods used to detect circulating antibodies against Friedländer's

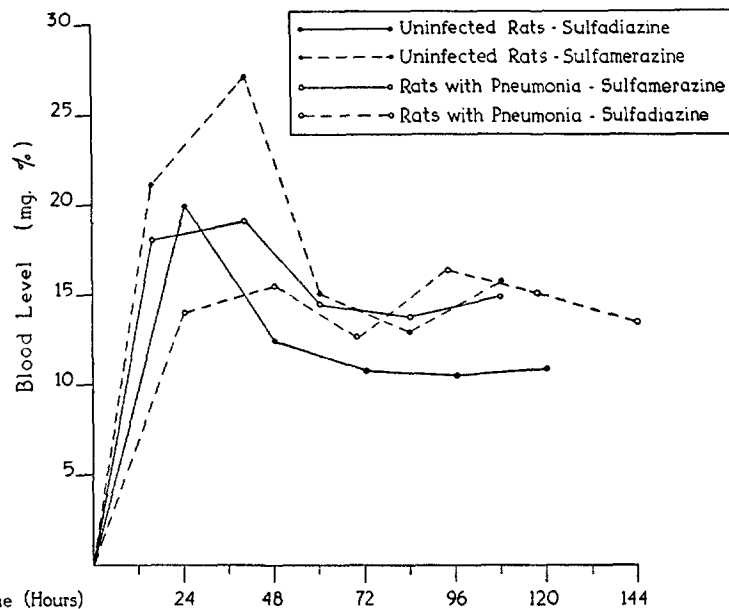
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bacillus included the standard mouse protection test (16) and a microscopic test for opsonins and agglutinins. In the mouse protection test 0.2 ml. of pooled serum (obtained from four to six rats) was injected intraperitoneally together with 0.2 ml. of tryptose broth containing 5 per cent para-aminobenzoic acid² and approximately 50 Friedländer's bacilli. Five mice were used in testing each lot of pooled serum, and the final results of the tests were tabulated at the end of 1 week. The procedure used in testing for opsonins and agglutinins was as follows. Washed Friedländer's bacilli obtained from 0.25 ml. of a 4 hour culture of the organisms in serum infusion broth were suspended in 0.1 ml. of the test serum, together with leucocytes obtained from peritoneal exudate of rats (17). The mixture was placed on a glass slide and incubated for 30 minutes in a sealed Petri dish lined with moist filter paper (17).



TEXT-FIG. 1. Sulfonamide blood levels in normal rats and rats with experimental pneumonia treated by mouth with sulfamerazine and sulfadiazine. Each point on the curves represents the average of values obtained on five or more animals. (For dosage, see text.)

At the end of the period of incubation smears were made and stained with methylene blue. Both phagocytosis and agglutination of extracellular organisms were considered in recording the final results of the tests on each lot of serum.

RESULTS

Effect of Sulfonamide Chemotherapy upon Fatality Rate.—When treatment with either sulfadiazine or sulfamerazine³ was begun 6 hours after inoculation more than 90 per cent of the animals survived the infection. As shown in

² To neutralize the bacteriostatic effect of the sulfonamide contained in the rat serum.

³ At no time was any appreciable difference noted in the effect of sulfadiazine and sulfamerazine upon the experimental infection.

Table I, there were two deaths from pneumonia among the 33 rats so treated. More than half of these animals were allowed to survive longer than 10 days before being sacrificed. When treatment was withheld until 9 hours after inoculation, the survival rate was appreciably lower, and after 12 hours only half of the animals survived the pneumonia. Treatment begun 18 hours after inoculation appeared to have no appreciable effect upon the course of the infection. In all subsequent treatment experiments sulfonamide therapy was started 6 hours after inoculation.

Effect of Sulfonamide Chemotherapy upon the Gross Pneumonic Lesion.—The action of sulfonamide chemotherapy upon the pulmonary lesion was studied in a series of treated rats sacrificed at 6, 18, 24, 36, 42, 66, 90, and 168 hours after the start of treatment. Three rats were killed at each interval, and the lungs were examined in the gross before being fixed for histological study (Text-fig. 2).

TABLE I
Survival Rate among Rats with Experimental Friedländer's Pneumonia Treated with Sulfonamide at Various Intervals after Inoculation

Time after inoculation that treatment was begun	No. of rats treated	No. of rats surviving pneumonia	Survival
<i>hrs.</i>			<i>per cent</i>
6	33	31	94
9	19	14	73
12	10	6	60
18	9	0	0







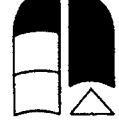
Six hours after the start of treatment the gross lesion occupied about half the left lung and was bright red in color. The border between the normal and consolidated lung was hemorrhagic and irregular, presenting the same appearance as in untreated animals 12 hours after inoculation (13). Both the size of the lesion and the character of its border indicated that the drug had exerted little or no effect upon the spreading pneumonia during the first 6 hours of treatment. At 18 hours the lesion was slightly larger, but its margin was now sharp and lacked the irregular hemorrhagic appearance noted at 6 hours. Thereafter there occurred no further spread of the pneumonia, and at 24, 36, and 42 hours the lesion became grayer and more firm in consistency. After 66 and 90 hours of treatment the area of consolidation appeared to be slightly smaller than at 18 hours, and definite contraction of the hard gray lesion was noted at the end of 1 week. At each of the above intervals the site of the original mucin inoculation was identifiable as a dark red area which differed in color from the pneumonic lesion and failed to spread.

In marked contrast to the pathological findings in untreated animals (13) neither pleurisy nor pericarditis was noted in rats sacrificed during sulfonamide therapy.

Effect of Chemotherapy upon Bacteremia.—Frequent blood cultures were taken during the course of treatment; the results are recorded in Text-fig. 2. After 6 hours of treatment bacteremia was present in more than 50 per cent of the

animals. At no later time, however, did the incidence exceed 15 per cent, and after 1 week's treatment all blood cultures were sterile.

Histopathology of Pulmonary Lesion in Treated Animals.—Tissue sections were studied from the lungs of all of the rats sacrificed at the intervals stated in Text-fig. 2. Additional rats were also killed at 3, 18, and 24 hours after the start of treatment, and the lungs of three animals at each interval were examined histologically to determine the effect of treatment upon the lesion.

Time after Treatment	6 hours	18 hours	42 hours	66 hours	90 hours	1 week	Untreated Rats ^{**}
Approximate Size of Pulmonary Lesion [*]							
Blood Culture Positive	7	2	0	2	1	0	5
Blood Culture Negative	6	11	13	12	14	13	0
Incidence of Bacteremia	54%	15%	0%	14%	7%	0%	100%

^{*} Based on autopsy findings in 3 rats killed at each interval

^{**} Untreated rats died in less than 100 hours

TEXT-FIG. 2. Effect of sulfonamide chemotherapy upon pulmonary lesion and incidence of bacteremia in experimental Friedländer's pneumonia. Treatment begun 6 hours after inoculation.

As shown in Fig. 1, the morphology of the Friedländer bacilli in the outer edema zone at the end of 3 hours of therapy appeared to be normal. Examination of the rest of the lesion revealed it to be identical with the early pneumonia observed in untreated animals. After 6 hours of treatment, the edema zone was still prominent (Fig. 2), but the Friedländer bacilli in the edema-filled alveoli were now noted to be enlarged, pleomorphic, and irregularly stained (Fig. 3), indicating bacteriostasis (18). At 18 hours the edema zone was no longer present at the margin of the lesion (Fig. 4), and the pneumonic process appeared to have stopped spreading. In the outermost alveoli, where the bacteria were most plentiful, at both 18 and 24 hours, organisms could be seen in the cytoplasm of the polymorphonuclear leucocytes (Fig. 5). After 36 hours of treatment phagocytosis of the bacteria appeared to be more complete (Fig. 6), and at later intervals large mononuclear cells could be seen to be taking part in the phagocytic process (Fig. 7). At the end of 90 hours the alveolar exudate was predominantly mononuclear; extracellular bacteria were now no longer visible and most of the intracellular organisms appeared to have been digested by the phagocytes (Fig. 8). Signs of clearing of the pneumonic process were prominent at the end of the 7 day period of treatment.

The Effect of Sulfonamide Therapy upon the Formation of Abscesses in the Lung.—The lungs of a few of the treated rats exhibited definite lung abscesses

at the time of autopsy. The incidence of abscess formation was higher in animals treated 12 hours after inoculation than in those treated at 6 hours. In one experiment treatment was begun 6 hours after inoculation and was maintained for a period of 2 weeks. Two rats so treated and sacrificed on the 35th day had small but definite lung abscesses from which Friedländer's bacilli were recovered in pure culture.

The Relation of Phagocytosis in the Lung to the Presence of Circulating Antibody.—The serum from sulfonamide-treated rats recovering from Friedländer's bacillus pneumonia was tested for mouse-protective, opsonizing, and agglutinating antibodies at the end of the 1st, 2nd, 3rd, 4th, and 7th days of treatment. Although phagocytosis was prominent in the lungs within the first 24 hours of treatment, no antibody could be detected in the blood serum until after 4 or more days of treatment (see Table II).

TABLE II

Tests for Antibody in Serum of Rats with Experimental Friedländer's Pneumonia Treated with Sulfonamide

Duration of pneumonia <i>hrs.</i>	Mouse protection test*	Opsonocytophagic test	Agglutination test
24	0-5	0	0
48	1-5	0	0
72	0-5	0	0
96	0-5	±	0
168	3-5	+	+

* First figure indicates number of mice surviving at end of 1 week; second figure refers to number of mice inoculated.

DISCUSSION

Friedländer's bacillus pneumonia induced in white rats by the methods described in the present studies (and not treated) constitutes an acute pulmonary infection which is almost uniformly fatal. Although somewhat more fulminating in its course than human Friedländer's pneumonia, the experimental murine infection resembles very closely the acute form of the human disease (13). The experiments here reported show that the experimental pneumonia in rats, in spite of its fulminant character, can be controlled by adequate sulfonamide therapy provided the drug is administered early in the disease. The manner in which the drug exerts its beneficial effect is revealed by the histology of the recovery process in the lung.

In the untreated animal Friedländer's bacilli appear to cause consolidation of the lung by the same mechanism as does the pneumococcus (15). The organisms can be seen in large numbers in the outer edema zone of the lesion where they are carried mechanically into adjacent alveoli by the edema fluid, thus

producing a spread of the lesion. As in experimental pneumococcal pneumonia (19) sulfonamide chemotherapy appears to exert its main effect in this spreading edema zone. Six hours after the start of treatment the Friedländer bacilli in the edema-filled alveoli exhibit morphological changes indicative of bacteriostasis (18), and shortly thereafter the pneumonic lesion ceases to spread. The appearance of edema fluid in the outermost portion of the spreading lesion constitutes the earliest stage of inflammation in the infected alveoli. When the lesion stops spreading as the result of bacteriostasis, phagocytes accumulate in all of the infected alveoli that previously contained only edema fluid, and since no further spread of the infection occurs, the edema zone disappears from the margin of the lesion. Once phagocytes have accumulated in sufficient number in the infected alveoli, they destroy the bacilli by the same phagocytic process that operates in the zone of consolidation in untreated animals (13). The sulfonamide drug brings about recovery by inhibiting the growth of the bacteria at the advancing margin of the lesion, thereby enabling the alveolar phagocytes to destroy the bacteria that have invaded the lung. Only in areas of abscess formation do the phagocytes fail to destroy all of the offending organisms.

Although from these studies little doubt appears to exist as to the manner in which sulfonamide chemotherapy causes recovery in Friedländer's pneumonia, the exact mechanism involved in the phagocytosis and ultimate destruction of the bacteria remains obscure. Friedländer's bacillus, like pneumococcus, possesses a protective capsule which renders the organism resistant to phagocytosis. Why then should phagocytosis occur in the pneumonic lesions of both treated and untreated animals? In the case of experimental pneumococcal pneumonia (18), phagocytosis is observed in the first 24 hours of the infection and occurs even in the lungs of bacteremic animals. Thus it appears unlikely that circulating antibody is responsible for the phagocytic reaction. Likewise, in the present experiments, no antibody could be detected in the blood of rats recovering from Friedländer's pneumonia until several days after phagocytosis had begun in the lungs. All of the evidence thus far advanced indicates that the ultimate recovery of animals with Friedländer's pneumonia when given sulfonamide depends upon the phagocytosis of the offending bacteria, and yet no satisfactory explanation can be given for the occurrence of phagocytosis of the encapsulated organisms in the absence of opsonins. Therefore, further experiments have been carried out to elucidate the exact mechanism of this non-antibody phagocytic reaction in the lung, and to determine the reason for its failure to operate efficiently in areas of abscess formation. The results of these experiments are reported in the following paper.

SUMMARY

Sulfonamide chemotherapy was found to cure rats of an otherwise fatal form of experimental Friedländer's bacillus pneumonia when treatment was begun 6

hours after inoculation. Most of the pneumonic lesions cleared completely, but an occasional animal exhibited small residual abscesses in the previously consolidated lung.

The recovery process taking place in the lungs was studied histologically at various intervals during therapy. As in the case of pneumococcal pneumonia, the principal action of the sulfonamide was upon the bacteria in the advancing edema zone at the periphery of the pneumonic lesion. The bacteriostatic action of the drug appeared to stop the spread of the pneumonia, and the Friedländer bacilli were ultimately ingested and destroyed by the phagocytic cells in the alveolar exudate.

The phagocytosis of bacteria in the lung was shown to be unrelated to the presence of antibody in the blood.

BIBLIOGRAPHY

1. Buttle, G. A. H., Parish, H. J., McLeod, M., and Stephenson, D., *Lancet*, 1937, **1**, 681.
2. Gross, P., Cooper, F. B., and Lewis, M., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 12.
3. Bliss, E. A., Feinstone, W. H., Garrett, A. W., and Long, P. H., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 619.
4. Kolmer, J. A., and Rule, A. M., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 305.
5. Feinstone, W. H., Williams, R. D., Wolf, R. T., Huntington, E., and Crossley, M. L., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 427.
6. Julianelle, L. A., *Ann. Int. Med.*, 1941, **15**, 190.
7. Sesler, C. L. and Schmidt, L. H., *J. Pharmacol. and Exp. Therap.*, 1943, **79**, 117.
8. Sinclair, F., *Brit. Med. J.*, 1941, **1**, 196.
9. Perlman, E., and Bullova, J. G. M., *Arch. Int. Med.*, 1941, **67**, 907.
10. Hyde, L., and Hyde, B., *Am. J. Med. Sc.*, 1943, **205**, 660.
11. Solomon, S., *J. Am. Med. Assn.*, 1940, **115**, 1527.
12. Trevett, G. I., Nelson, R. A., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1941, **69**, 303.
13. Sale, L., Jr., and Wood, W. B., Jr., *J. Exp. Med.*, 1947, **86**, 239.
14. Loosli, C. G., *Arch. Path.*, 1937, **24**, 743.
15. Wood, W. B., Jr., *J. Exp. Med.*, 1941, **73**, 201.
16. White, B., *The Biology of Pneumococcus*, New York, The Commonwealth Fund, 1938, 648.
17. Wood, W. B., Jr., Smith, M. R., and Watson, B., *J. Exp. Med.*, 1946, **84**, 387.
18. Stephenson, M., *Bacterial Metabolism*, London, Longmans, Green and Co., 1939, 216.
19. Wood, W. B., Jr., and Irons, E. N., *J. Exp. Med.*, 1946, **84**, 365.

EXPLANATION OF PLATES

Tissue sections from the lungs of rats with experimental Friedländer's bacillus pneumonia treated with sulfonamide. Fig. 5 was photographed by Mr. Cramer Lewis; the remaining figures by Mr. Milton K. Ehtold. All sections were stained by the Gram-Weigert technique.

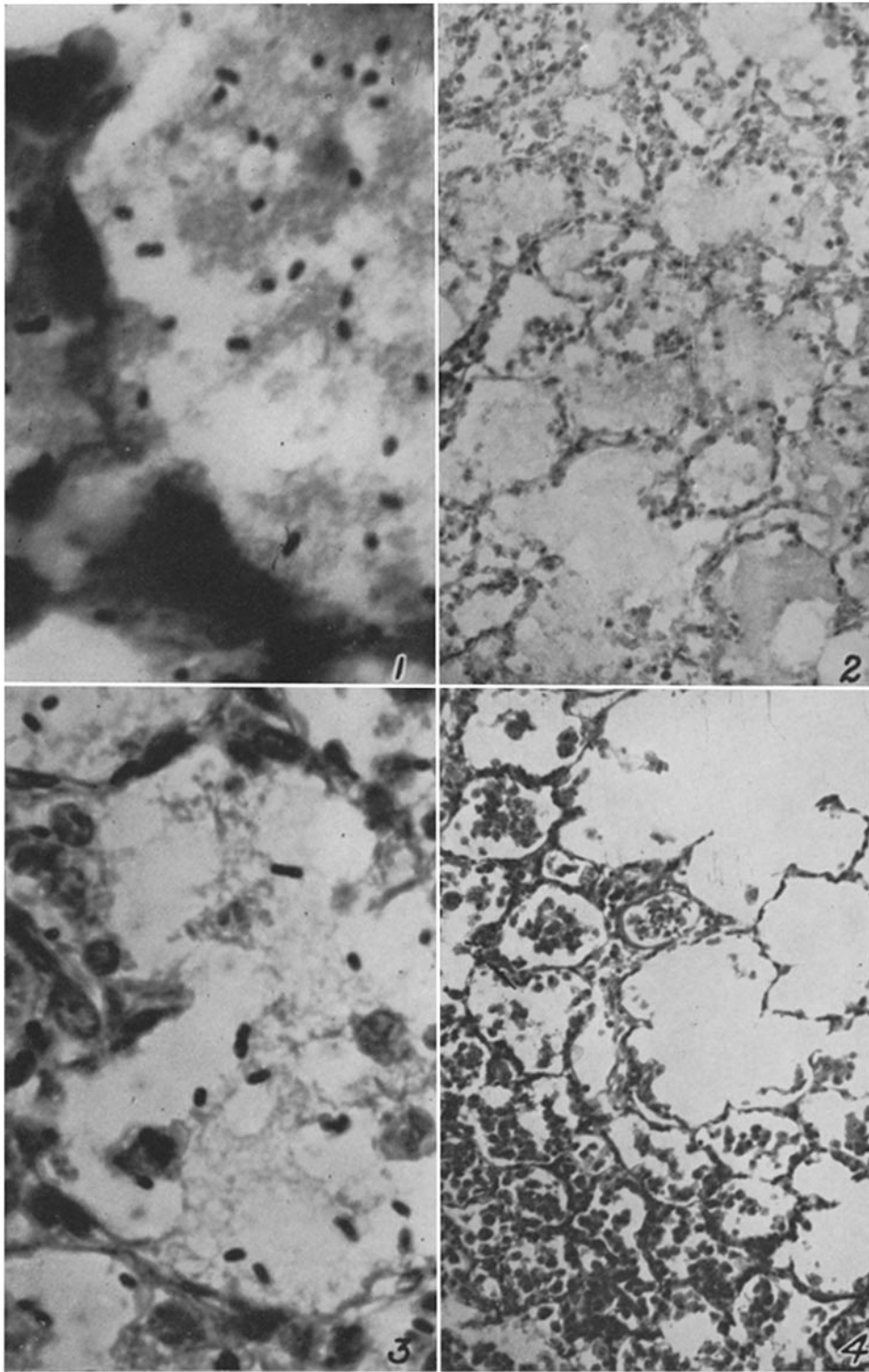
PLATE 25

FIG. 1. Bacteria-laden edema fluid in alveolus at margin of spreading lesion 3 hours after the start of treatment. The bacteria as yet exhibit none of the characteristic morphological signs of bacteriostasis. $\times 1500$.

FIG. 2. Edema zone still present at margin of lesion 6 hours after the start of treatment. $\times 200$.

FIG. 3. Pleomorphism of Friedländer's bacilli, indicating early bacteriostatic effect after 6 hours of treatment. $\times 1500$.

FIG. 4. Disappearance of edema zone from the margin of lesion 18 hours after beginning of treatment. $\times 200$.



(Sale *et al.*: Mechanism of recovery in pneumonia. II)

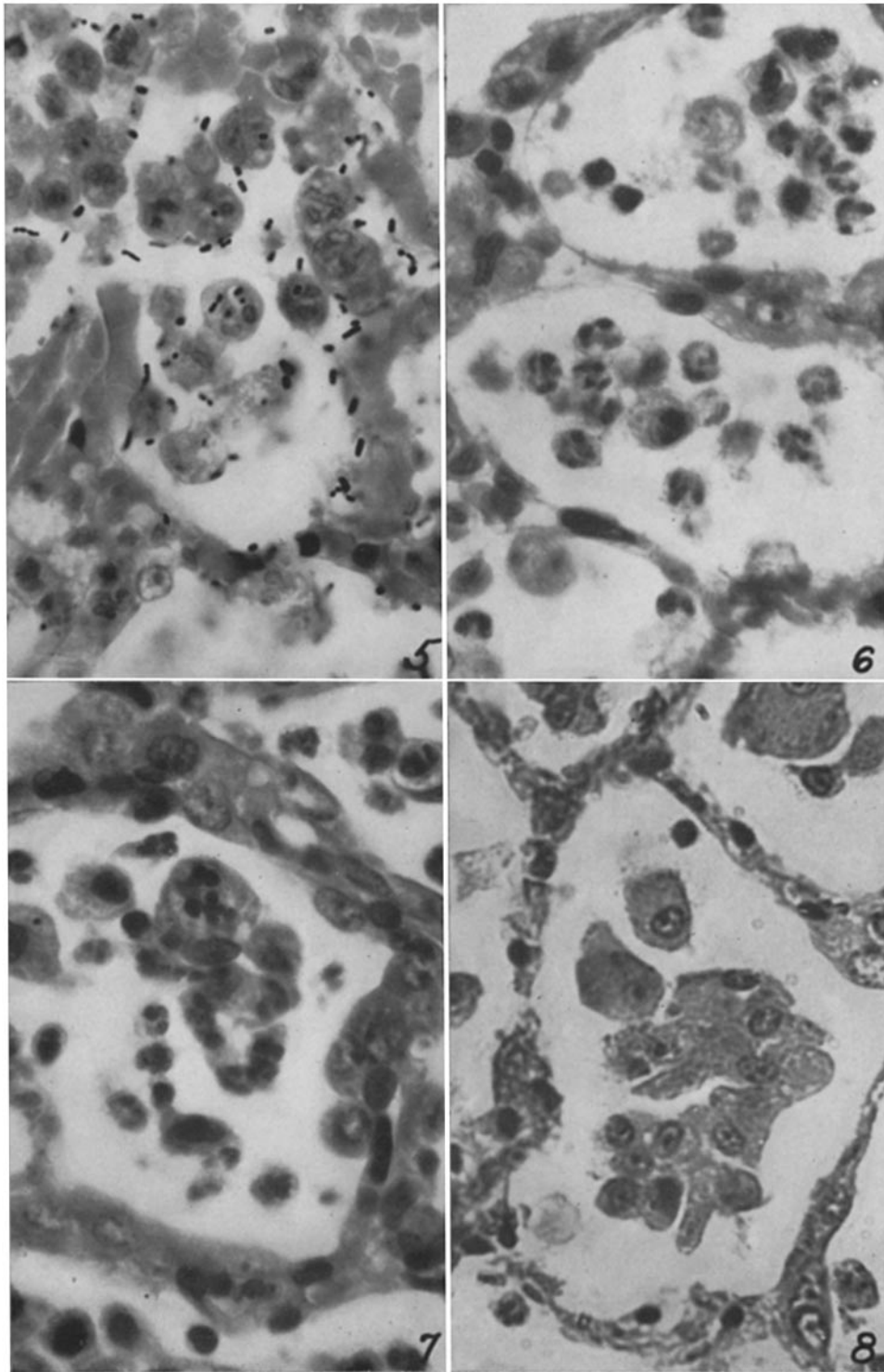
PLATE 26

FIG. 5. Early phagocytosis of Friedländer's bacilli in rat sacrificed at end of 24 hours of treatment. $\times 1500$.

FIG. 6. More complete phagocytosis of Friedländer's bacilli by leucocytes in alveolar exudate after 36 hours of treatment. $\times 500$.

FIG. 7. Macrophage reaction with a few intracellular organisms. This animal was sacrificed 66 hours after the start of treatment; the macrophage is the predominant cell. $\times 855$.

FIG. 8. Lesion after 1 week of treatment showing marked clearing with only macrophages in the alveoli. Bacteria are no longer visible in the cytoplasm of the phagocytes. $\times 1000$.



(Sale *et al.*: Mechanism of recovery in pneumonia. II)