THE EFFECT OF CORTISONE AND ANTIBIOTIC AGENTS ON EXPERIMENTAL PULMONARY ASPERGILLOSIS *

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For many years pathologists have encountered rare instances of human infection with classes of fungi that are widely prevalent in the environment but are generally regarded to be saprophytic. These infections are seldom encountered except in persons who are seriously debilitated as a result of other disorders. An increasing number of such human infections with "saprophytic" fungi such as Aspergillus, Mucor, and Candida have been reported recently.[1-5] These have occurred chiefly, as in the past, in chronically ill patients but particularly in patients who have been treated with cortisone or ACTH and with broad spectrum antibiotic agents during some stage of their ailments.

During the past 3 years, 18 patients with secondary fungal infections of the lungs have been observed on the Tulane Autopsy Service at Charity Hospital of Louisiana in New Orleans.[6] The causative organisms were Aspergillus, Candida, and Mucor (or Rhizopus), and most of these patients had been treated with cortisone or ACTH and antibiotic agents. In contrast, during the preceding 5-year period, only 4 similar instances of pulmonary infection were observed.

Some insight into the mechanisms by which antibiotic agents and cortisone might increase host susceptibility to fungal infections has been gained by experimental studies. It appears that antibiotic drugs can exert such an effect by acting on either the fungi themselves,[7-9] the host,[10,11] or both. Cortisone, however, is believed to act chiefly by inhibiting certain of the host defenses against infection.[12]

Our own observations of secondary fungal infections of the lungs in chronically ill patients treated with steroid hormones or antibiotic agents led us to investigate the possible effects of these substances on the susceptibility of mice to pulmonary infection by air-borne fungi. Under similar circumstances, altered susceptibility to Aspergillus,[13] Mucor,[14,15] and Candida[13,16-22] has been shown to exist in experimental animals. In most instances the method of inoculation with the fungus has not simulated conditions of natural exposure. In our experiments

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mice were treated with cortisone and antibiotic drugs and then subjected to inhalation of aerosols containing dry, viable spores of *Aspergillus flavus*. This method of exposure probably simulates more closely the mechanism by which human beings acquire fungal infections of the lungs.

Experimental Method

Mice are normally resistant to infection with aspergillus and other "saprophytic" fungi. Hence they are suitable animals for testing the possible role of cortisone and antibiotic drugs in reducing resistance. White female mice of the Carworth Farms CF1 strain were used in all experiments except one, in which male Taconic Farms mice were used. The mice were inoculated subcutaneously with 5 mg. of an aqueous suspension of cortisone acetate (Cortone Acetate, Merck Sharp & Dohme) and intramuscularly with 30,000 units of long-acting penicillin (Bicillin,® Wyeth) two days before exposure to spores of *Aspergillus flavus*. Another antibiotic agent, tetracycline hydrochloride (Achromycin,® Lederle) was added to the water (5 mg. per 100 ml.) which the animals drank freely throughout the course of the experiment. At least two control groups were investigated simultaneously. One received spores but no cortisone or antibiotic agents, and the other received cortisone and antibiotic agents but no spores.

The exposure chamber consisted of a closed bell jar containing a cylindrical wire mesh (Fig. 1). One outlet was available to provide fresh air when necessary and another for escape of excess air. Spores, obtained by vacuum suction from dried cultures of *Aspergillus flavus*, were sprayed into the chamber with a powder atomizer. The strain used was originally cultured from a human lung which was the seat of aspergillosis.

Mice were exposed to low, medium, or high concentrations of spores for 10 to 30 minutes and were observed for 3 weeks or until death. In some experiments designed to study the sequence of the pathologic lesions and the fate of the inhaled spores, mice were sacrificed at intervals following exposure.

The approximate number of spores retained in the lungs was determined by sacrificing 2 or 3 mice of each group immediately after exposure. The left lungs were removed and individually homogenized in sterile saline. Plate counts were then performed using suitable dilutions of the homogenates. Mice exposed to 3 different concentrations of spores retained approximately 360,000, 60,000 or 24,000 viable spores per left lung.

In order to determine whether cortisone and antibiotic agents given separately and in combination influenced the susceptibility of mice to

lethal pulmonary aspergillus infection, two experiments were performed (Table I). In each, mice were divided into 4 experimental groups, all of which were exposed to high concentrations of spores. One group received cortisone and antibiotic agents, a second received cortisone alone, a third received antibiotic drugs, and a fourth received no supplementary treatment.

TABLE I

Seven-Day Mortality in Mice Inhaling Aspergillus flavus Spores

No. of mice in group	Treatment			Mortality at end of 7 days
	Spores*	Cortisone†	Antibiotic agents‡	
16	+	+	+	14/16 (88%)
15	+	+	o	10/15 (67%)
7	+	o	+	0/7 (0%)
22	+	o	o	0/22 (0%)
20	o	+	+	0/20 (0%)
20	o	+	o	0/20 (0%)
7	o	o	+	0/7 (0%)
20	o	o	o	0/20 (0%)

* Approximately 360,000 viable spores retained per left lung.

† 5 mg. cortisone acetate, 2 days before exposure to spores.

‡ 30,000 units Bicillin,® 2 days before exposure to spores; tetracycline hydrochloride, 5 mg. per 100 ml. in water bottles.

One experiment was performed to learn whether the inhalation of a large number of dead spores would produce a pneumonitis. Mice with and without treatment by cortisone and antibiotic agents were exposed to spores previously heated at 103° C. for 24 hours. Their nonviability was confirmed by failure to grow on an agar medium. Some mice were sacrificed at intervals up to one week and others were observed for 10 weeks.

Two experiments were designed to determine whether multiple doses of cortisone would increase susceptibility to infection with lower concentrations of spores. In these experiments the mice were given injections of cortisone (2.5 mg.) at intervals throughout the 2 to 3 week period of observation in addition to the regular pretreatment with 5 mg. of cortisone 2 days before exposure. In one experiment (# 323, Table III) the mice inhaled a low concentration of spores and were given supplementary injections of cortisone at 2, 5, 8, 11, 14, and 17 days after exposure to the spores. In a second experiment (# 321, Table III) the animals inhaled a medium concentration of spores and the supplementary injections of cortisone were given 2 and 5 days after exposure.

All animals were necropsied. Portions of lung were cultured, and other portions were fixed in 10 per cent formalin. Sections were stained with hematoxylin and eosin and with the Gridley stain.[23]

Results

No mice died after treatment with cortisone alone, antibiotic agents alone, or a combination of the two. No mice died after receiving spores alone or in combination with antibiotic drugs. When mice treated with cortisone or with cortisone and antibiotic agents were exposed to spores, a high mortality resulted. Table I summarizes the data obtained when cortisone and antibiotic therapy were given individually or in combination to mice exposed to a high concentration of spores. The group that received cortisone and antibiotic drugs had an 88 per cent mortality by the seventh day while the group that received cortisone had a 67 per cent mortality at 7 days. This difference is not statistically significant. In this experiment and those mentioned next, no deaths occurred in any of the animals beyond the seventh day, and any mice which survived beyond 7 days were apparently healthy at the end of the 3 week period of observation.

The results obtained in 3 experiments in which mice were exposed to 3 different concentrations of spores are presented in Table II. No mice died after treatment with cortisone and antibiotic agents alone or after receiving spores alone. When mice treated with cortisone and antibiotic drugs were exposed to spores, a high mortality resulted, the rate being dependent upon the dose of spores. At 7 days, mice exposed to the high concentration of spores had an 88 per cent mortality; mice exposed to the medium concentration, a 50 per cent mortality, and mice exposed to the lowest concentration, a 30 per cent mortality.

The lungs from all of the control animals in these experiments were normal in appearance at the time of sacrifice, 3 weeks after inhaling spores. The lungs from the experimental mice which died following exposure to spores and treatment with cortisone and antibiotic agents were diffusely hyperemic, the seat of consolidation, and contained numerous hemorrhagic areas. Extensive hemorrhagic bronchopneumonia was demonstrable microscopically (Fig. 2). In Gridley stained sections masses of hyphae could be seen filling the lumens of small bronchi and invading through bronchial walls into the neighboring soft tissue and blood vessels. In the blood vessel lumens, thrombi made up of fibrin and a feltwork of interlacing hyphae were often evident. The hyphae appeared to breech anatomic barriers in their spread and often extended indiscriminately through such structures as bron-

chial cartilage and arterial walls (Figs. 3 and 4). The infiltration by hyphae in many instances failed to elicit any detectable tissue response. In complete necropsy examinations on mice with extensive hemorrhagic bronchopneumonia and numerous mycelial thrombi in pulmonary blood vessels, evidence of extrapulmonary embolic spread of the fungus was not encountered.

In the two experiments in which mice with and without cortisone

TABLE II

The Effect of Cortisone and Antibiotic Drug Administration on Fatal Pulmonary Aspergillosis in Mice Inhaling Varying Concentrations of Aspergillus flavus Spores

Experiment and group no.	Treatment: Spores*	Cortisone†	Antibiotic agents‡	No. of mice	Day of death after exposure: 1	2	3	4	5	6	7	Mortality at end of 7 days
320 A	Low dose	+	+	10			1	1			1	3/10 (30%)
B	Low dose	o	o	10								0/10 (0%)
C	o	+	+	8								0/8 (0%)
D	o	o	o	10								0/10 (0%)
318 A	Medium dose	+	+	8					4			4/8 (50%)
B	Medium dose	o	o	9								0/9 (0%)
C	o	+	+	9								0/9 (0%)
D	o	o	o	10								0/10 (0%)
317 A	High dose	+	+	8	3	3					1	7/8 (88%)
B	High dose	o	o	8								0/8 (0%)
C	o	+	+	10								0/10 (0%)
D	o	+	o	10								0/10 (0%)
E	o	o	o	10								0/10 (0%)

* Low dose: approximately 24,000 viable spores per left lung; medium dose: approximately 60,000 viable spores per left lung; high dose: approximately 360,000 viable spores per left lung.

† 5 mg. of cortisone acetate 2 days before exposure to spores.

‡ 30,000 units of Bicilllin,® 2 days before exposure to spores; tetracycline HCl, 5 mg. per 100 ml., in water bottles.

and antibiotic treatment were exposed to high concentrations of spores and sacrificed at varying intervals, the following observations were made. Mice from both groups sacrificed immediately after exposure to spores showed no lung lesions. However, on microscopic examination many spores were seen on the mucosal cells of bronchi and bronchioles. These were unaccompanied by inflammatory reaction. Mice of both groups sacrificed one day after exposure were found to have bronchitis and early bronchopneumonia (Fig. 5). The only detectable difference was that spores alone were seen in the lungs of the control animals,

while in the lungs of the experimental animals hyphae as well as spores were present. This observation suggests that the cortisone and antibiotic treatment may have an effect on the organisms as well as the host. After 4 days the bronchopneumonia was found to be undergoing resolution and many macrophages were present in the lungs of the control mice (Fig. 6). Spores, many within macrophages, were readily identified, but no hyphae were present. At this time the lungs of the experimental mice showed an extensive hemorrhagic bronchopneumonia (Fig. 7). Hyphae were seen throughout, and no macrophages were demonstrable in the pulmonary exudate. At 7 days the lungs of the control animals were essentially normal whereas 88 per cent of the experimental animals had died. In the lungs of the control mice, spores could be identified in the tissue and in macrophages until the seventh day, and even though they were not detected microscopically after longer time intervals, a few could be cultured after two weeks. In no instance were hyphae observed in the lungs of the control mice.

In the experiment in which mice were exposed to massive clouds of heat-killed spores, no animals died, either in the group treated with cortisone and antibiotic agents or in the untreated group. No gross or microscopic lesions were observed in the mice sacrificed 4 or more days after inhaling spores. In mice of both groups sacrificed 1 to 3 days after exposure, spores were seen in the bronchi, but no cellular response was manifest.

Table III summarizes the results of the two experiments which were designed to determine whether continuation of cortisone treatment after inhalation of spores would further increase susceptibility. Mice inhaling the low concentration of spores (Experiment 323) and receiving multiple injections of cortisone continued to die throughout the 3 week period of observation, with a final mortality of 86 per cent. Among those which inhaled the medium concentration of spores (Experiment 321), the cumulative mortality was 92 per cent. These results are in contrast to the much lower mortality observed in the mice receiving only one injection of 5 mg. of cortisone 2 days before exposure to approximately the same low or medium concentration of spores (Table II). The pathologic lesions in these experiments were similar to those described in the preceding studies.

Discussion

The experiments indicate that normal mice can inhale large numbers of spores of *Aspergillus flavus* and suffer only a mild self-limiting bronchitis and pneumonitis. However, pretreatment with cortisone

TABLE III

The Influence of Multiple Doses of Cortisone Acetate on Fatal Pulmonary Aspergillosis in Mice Exposed to Low or Medium Concentrations of Aspergillus flavus Spores

Experiment and group no.	Treatment			No. of mice	Day of death after exposure																			Mortality at end of 3 weeks
	Spores*	Cortisone	Antibiotic agents†		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
323 A	Low dose	+‡	+	14				1	3			1						1	1	1	1	1			2	12/14 (86%)
B	Low dose	0	0	13																						0/13 (0%)
C	0	+‡	+	15														2								2/15 (13%)
D	0	0	0	12																						0/12 (0%)
321 A	Medium dose	+§	+	13	1	1			4	1	3	1		1												12/13 (92%)
B	Medium dose	0	0	16																						0/16 (0%)
C	0	+§	+	13																						0/13 (0%)
D	0	0	0	8																						0/8 (0%)

* Low dose: approximately 24,500 viable spores per left lung; medium dose: approximately 49,000 viable spores per left lung.

† 30,000 units of Bicillin,® 2 days before exposure to spores; tetracycline HCl, 5 mg. per 100 ml., in water bottles.

‡ 5 mg. of cortisone acetate, 2 days before exposure to spores, and 2.5 mg. cortisone acetate on 2nd, 5th, 8th, 11th, 14th and 17th days after exposure.

§ 5 mg. cortisone acetate 2 days before exposure to spores, and 2.5 mg. cortisone acetate on 2nd and 5th days after exposure.

and antibiotic drugs renders the mice highly susceptible to fatal pulmonary aspergillosis. It is apparent from the results listed in Table I that cortisone rather than the antibiotic treatment is chiefly responsible for the enhanced susceptibility.

Two independent observations suggest that even extremely low concentrations of air-borne spores may be sufficient to induce severe pulmonary infection in occasional cortisone-treated animals. Sági and Lapis,[24] while treating rats with cortisone and studying tumor transplants, found that many of their animals died with pulmonary aspergillosis. They attributed this to the cortisone treatment. Moreover, in our own experiments, on one occasion a leak developed in the exposure chamber and a small number of spores escaped into the room air. Subsequently, several mice in a control group which had received cortisone and antibiotic agents but no spores died (animals originally in Experiment 321 C, Table III, but not listed). At necropsy these animals were found to have pulmonary aspergillosis, attributed to infection with spores which had escaped into the air of the room.

The pathologic events disclosed by sacrificing mice at varying time intervals are of interest. The control animals which inhaled spores in large numbers developed a transient purulent bronchitis and bronchopneumonia within a day, but the pneumonia was resolved by the fourth day, and at 7 days the lungs were histologically normal. Spores but no hyphae were demonstrable within the lungs throughout this period. Though many of the spores remained viable for several weeks, as demonstrated by culture, they appeared to be unable to germinate into hyphae. The inhibition of germination may be attributed to some aspect of the host's natural resistance. Phagocytosis of the spores by macrophages may be one of many protective mechanisms involved. In animals exposed to spores and treated with cortisone and antibiotic drugs, a bronchitis and bronchopneumonia developed within a day. This was at first indistinguishable from that present in the controls. However, it progressed to a hemorrhagic bronchopneumonia and produced a high mortality within 7 days. In these animals hyphae developed within 24 hours and were soon seen invading throughout the lungs. Under the conditions of our experiments cortisone failed to inhibit the acute inflammatory response. This is in agreement with the observations of some workers[25,26] but contrary to those of others.[27-29] However, the macrophage response found in the untreated mice after a few days was not seen in any of the cortisone- and antibiotic-treated mice, either in those which died or those which were sacrificed. This indicates that the macrophage response in the lungs may be partly or completely

inhibited by cortisone. Such a conclusion is consistent with the observations of others.[27,29]

The gross and histologic lesions in the lungs of cortisone-treated mice exposed to spores of *Aspergillus flavus* closely resemble those found at necropsy in human cases of pulmonary aspergillosis.[6] Although terminal pulmonary aspergillosis is a relatively rare complication even in chronically ill patients, our experiments suggest that cortisone and antibiotic treatment may increase susceptibility to this and possibly other serious fungus infections in human subjects.

SUMMARY

Untreated mice and those receiving cortisone and antibiotic drugs were exposed to inhalation of aerosols containing dry, viable spores of *Aspergillus flavus*. Untreated mice and those given antibiotic agents and subsequently exposed to spores in large numbers developed only transient nonfatal pneumonitis. The administration of cortisone and antibiotic drugs or of cortisone alone rendered animals that inhaled spores highly susceptible to fatal pulmonary aspergillosis. The number of spores inhaled and the duration of cortisone treatment influenced the mortality.

Inhaled spores rapidly germinated into hyphae which penetrated throughout the lungs in mice treated with cortisone and antibiotic agents, but did not germinate into hyphae in the lungs of control mice. Heat-killed spores produced no evident lesions in control or experimental mice.

The increased susceptibility of cortisone- and antibiotic-treated mice to air-borne spores of *Aspergillus flavus* strengthens the hypothesis that a similar increase in susceptibility to air-borne saprophytic fungi may develop in certain chronically ill patients receiving these agents.

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[*Illustrations follow*]

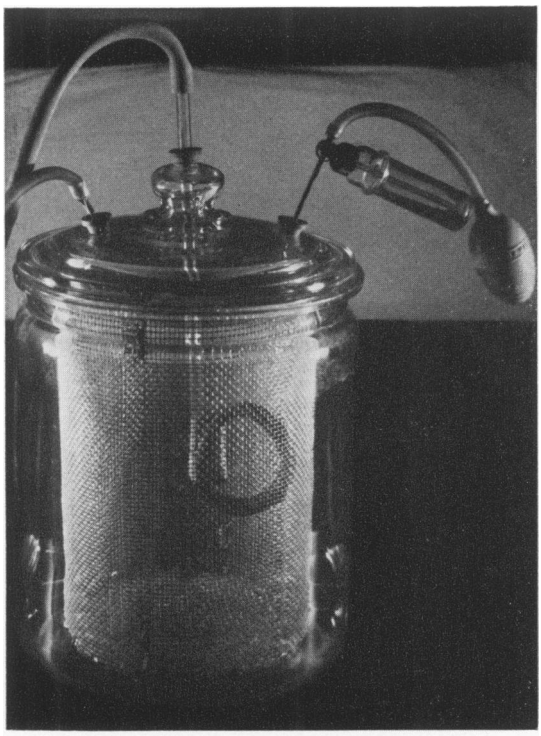
LEGENDS FOR FIGURES

FIG. 1. Exposure chamber consisting of a closed bell jar containing a cylindrical wire mesh. The powder atomizer used to spray dried spores is at upper right.

FIG. 2. Lung of mouse exposed to spores and treated with cortisone and antibiotic drugs, showing hemorrhagic bronchopneumonia with vascular thrombosis (lower left). Hematoxylin and eosin stain. × 100.

FIG. 3. Lung of mouse exposed to spores and treated with cortisone and antibiotic agents, showing hyphae invading through bronchus and into adjacent blood vessel (upper right). The double membrane in the vessel (upper right) is the elastic lamina. Gridley stain. × 55.

FIG. 4. Higher magnification of blood vessel in Figure 3, showing hyphae penetrating the elastic lamina. Gridley stain. × 900.



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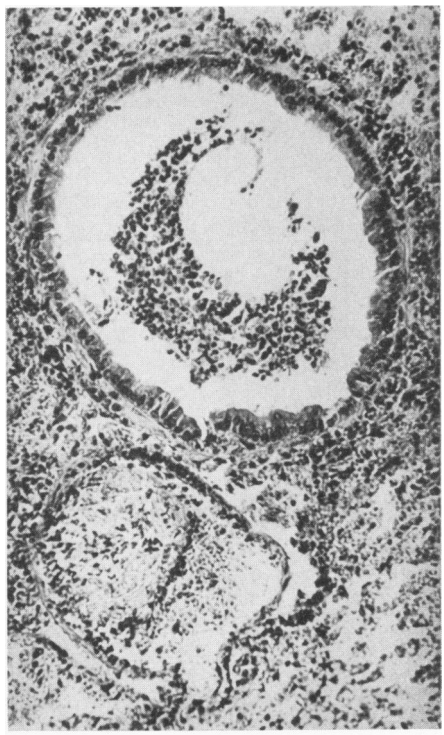

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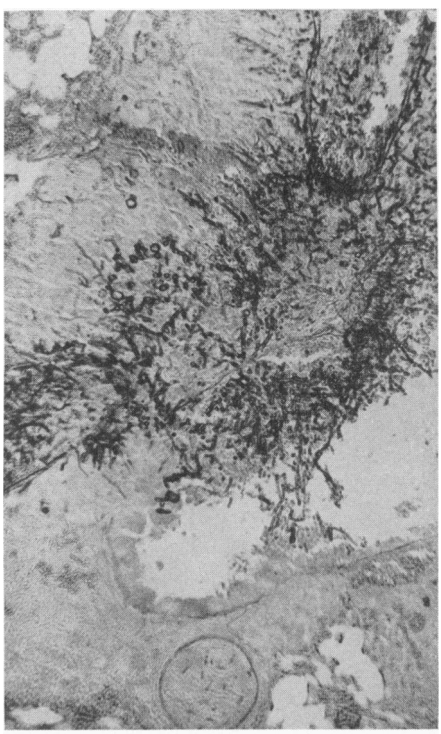

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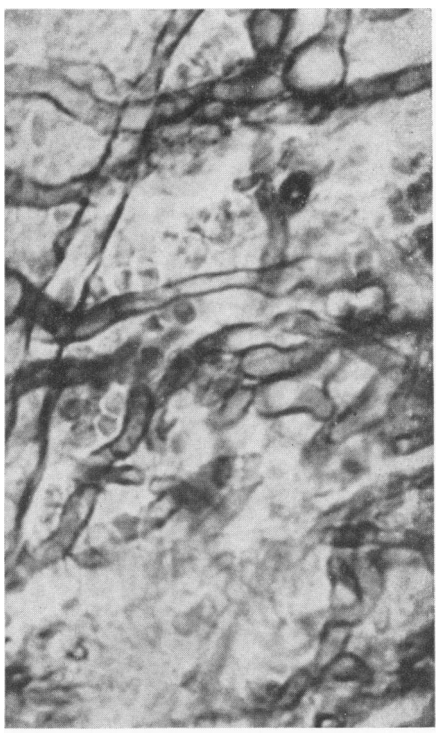

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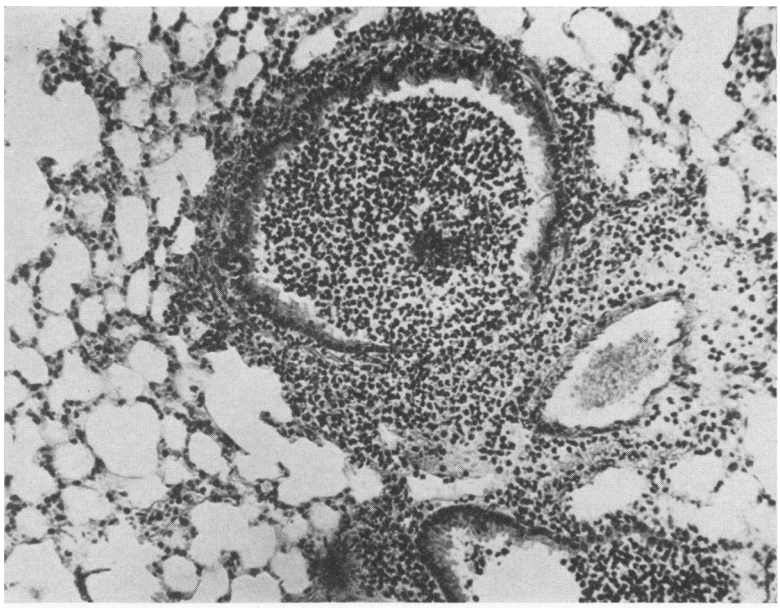
FIG. 5. Lung of mouse treated with cortisone and antibiotic drugs and sacrificed one day after exposure to aerosols containing viable spores. Lung shows acute bronchitis and early bronchopneumonia. Control mice at this time interval showed the same lesion. Hematoxylin and eosin stain. × 145.

FIG. 6. Lung of control mouse sacrificed 4 days after exposure to aerosols containing viable spores, showing extensive macrophage response surrounding a bronchus. Hematoxylin and eosin stain. × 145.

FIG. 7. Lung of mouse treated with cortisone and antibiotic agents and sacrificed 4 days after exposure to aerosols containing viable spores. There is extensive bronchitis and bronchopneumonia. An artery contains a thrombus (lower left). Hematoxylin and eosin stain. × 145.



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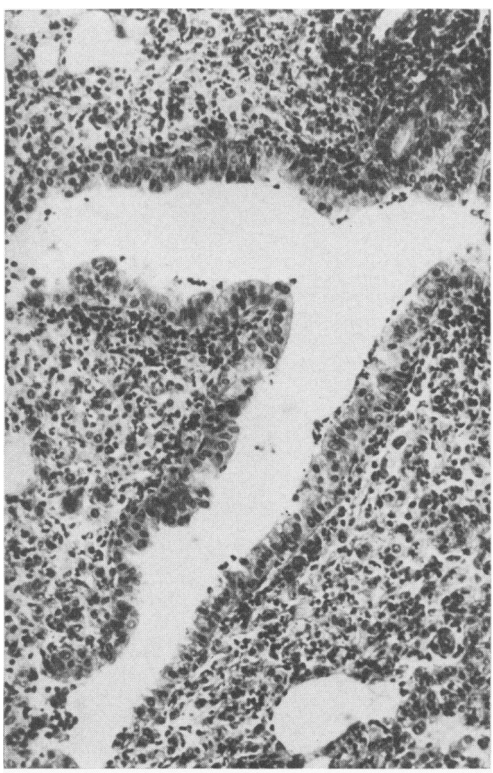

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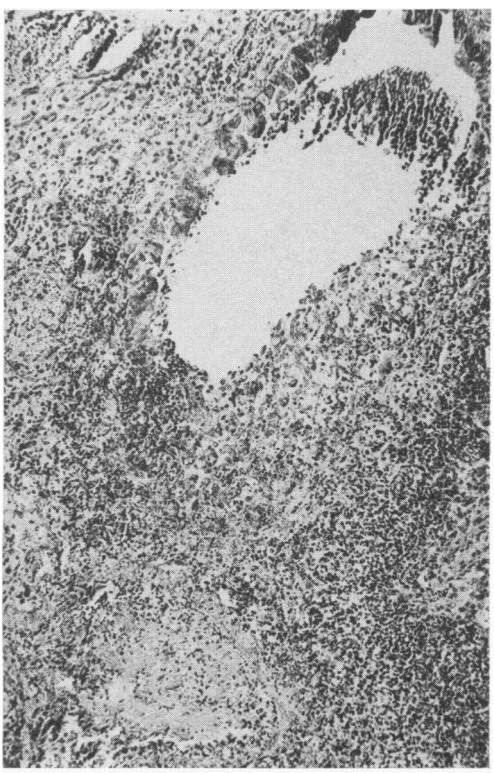

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