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Effect of Streptomycin Aerosol on Friedlander's Pneumonia in Rats.*

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(Introduced by Philip Hadley.)

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In view of the demonstration of the effectiveness of penicillin aerosol on experimental pneumococcus pneumonia in rats, reported previously by us,[1] it became of interest to ascertain the effect of streptomycin inhalations on an experimental pulmonary infection produced by gram-negative organisms. Favorable results following the use of streptomycin aerosol in human pulmonary infection have been reported by Olsen[2] in 9 cases of bronchiectasis, in the sputum from which gram-negative bacteria predominated; and by Menefee and Fogel[3] in 2 cases of Friedländer's infection. Human cases of Friedländer's pneumonia are rare, but very severe and nearly always fatal. Friedländer's bacillus (*Klebsiella pneumoniae*) is known to be sensitive to streptomycin. In experiments on mice infected intranasally with this organism Heilman[4] has shown that streptomycin, administered subcutaneously every 12 hours, beginning three hours after infection, had little effect in protecting them when the treatment covered a 3 day period, but that this dosage gave definite protection when treatment was continued for 7 days.

Since albino rats are known to be susceptible to infection with Friedländer's bacillus an experimental approach to the problem of the effectiveness of streptomycin inhalations on Friedländer's pneumonia was available, similar to that used in our pneumococcus study in which intrabronchially-infected rats were treated by exposure to penicillin-containing mist. The present report gives the results of 4 experiments in which rats, infected intrabronchially with mucin suspensions of Friedländer's bacillus, were treated successfully with inhalations of streptomycin aerosol. In two experiments comparison was made between the effect of intramuscular and inhalational administration of streptomycin.

Methods. Infection of rats. Rats weighing from 175 to 250 g were used. Through the courtesy of Dr. F. R. Heilman of the Mayo Clinic a Type A strain No. 837 of the Friedländer bacillus in the mucoid phase was obtained. This was highly virulent for mice in dilutions up to 10^{-6} cc by the intraperitoneal route and was found to be sensitive to streptomycin in a concentration of 0.5 μg/cc.

Preliminary intrabronchial inoculations of rats, by the technique previously described,[1] showed the lethal dose by this route to be 300 organisms (0.15 cc of a 10^{-7} dilution of a 16-hour broth culture) when suspended in 6% mucin. Intrabronchial doses of 50,000,000 organisms, suspended in *broth,* did not cause death. The survival time after one lethal dose of 300 organisms, in mucin, was approximately 150 hours. With larger doses the survival time decreased, 30,000,000 mucin-suspended organisms (100,000 M.L.D.) giving an average survival time of 50 hours. In the treatment experiments the dosage of mucin-suspended organisms ranged from 5,000,000 (17,000 M.L.D.) to 30,000,000 (100,000 M.L.D.).

Rats infected intrabronchially and dying from Friedländer pneumonia showed pulmonary lesions similar to those observed in this disease in man. The inoculated lobe was

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1 Hadley, F. P., McIlroy, A. P., and Laurent, A. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 204.

2 Olsen, A. M., *Proc. Mayo Clinic*, 1946, **21**, 53.

3 Menefee, E. E., and Fogel, D. H., *N. Carolina Med. J.*, 1947, **8**, 165.

4 Heilman, F. R., *Proc. Mayo Clinic*, 1945, **20**, 33.

greatly enlarged, light gray in color, and consolidated. Sections showed edema, acute pneumonitis, with necrotic areas. The other lobes showed an acute inflammation. All lobes and the heart were covered with a thick, slimy exudate, containing encapsulated organisms. Occasional rats developed lung abscess, with adhesions to the diaphragm and chest wall.

Treatment. The first treatment was given within 1 or 2 hours after intrabronchial inoculation of mucin-suspended organisms. For inhalational treatment 10 rats were placed together in the treatment chamber[1] into which the streptomycin-containing mist was introduced from a *Vaponephrin* nebulizer containing an 0.85% NaCl solution of the drug, in a concentration of 50,000 or 75,000 μg/cc. The apparatus and the method of calculating the amount of streptomycin inhaled by each rat were described in a previous report.[1] The average *estimated* amount of the drug inhaled by each rat in the different experiments ranged from 65 to 150 μg per minute, or a total of from 1,000 to 2,200 μg per 15 minutes of treatment. As in the penicillin aerosol experiments the *actual* dosage by the inhalational route was probably much lower than our calculated figures. The reasons for this are explained in the previous paper.

Inhalational treatment began 1 or 2 hours after infection, and continued on the first day at 3-hour intervals until 4 treatments had been given. Each treatment consisted of a 15-minute exposure to the mist. This schedule was followed on 3 or 4 successive days.

Rats receiving streptomycin by intramuscular injection were treated at the same intervals with 1,000 μg dissolved in 0.1 cc of physiological saline in Experiment I and with 500 μg in Experiment IV.

Results. Table I summarizes the results of the experiments. In Experiment 1 30 rats were inoculated intrabronchially with 30,000,000 (100,000 M.L.D.) mucin-suspended organisms. Beginning one hour after infection 10 rats received in a 3-day period a total of 11 intramuscular injections of 1,000 μg of the drug. All of these rats survived.

TABLE I.

Effect of Streptomycin, Administered Parenterally and by Inhalation, on Friedlander Pneumonia in Rats.

Exp. No.	No. of organisms inoculated	Treatment					Results			
		Route	No.	Days	Hr after infection	Total μg per rat × 1000	No. of rats	No. survived	% survival	Avg. surv. time (hr)
I	30,000,000 (100,000 M.L.D.)	Intramusc.	11	3	1 to 54	11	10	10	100	—
		Inhalation*	11	3	1 to 54	11	10	6	60	103
		Control	—	—	—	—	10	0	0	50
II	28,000,000	Inhalation*	15	4	1 to 77	15	10	7	70	78
		Control	—	—	—	—	10	0	0	54
III	8,000,000 (30,000 M.L.D.)	Inhalation†	16	4	1 to 78	25.6	10	7	70	128
		Control	—	—	—	—	10	0	0	69
		Intramusc.	16	4	2 to 79	8	5	5	100	—
		Inhalation†	16	4	2 to 79	35	10	10	100	—
IV	5,000,000 (17,000 M.L.D.)	Control	—	—	—	—	10	2	20	95

* 50,000 μg/cc salt solution. † 75,000 μg/cc salt solution.

In addition, 10 rats received 11 inhalational treatments with streptomycin aerosol over a 3-day period (estimated 1,000 μg per treatment). Six of these rats survived. All 10 untreated rats died from pneumonia in an average time of 50 hours. The average survival time of the 4 rats which died, despite inhalational treatment, was 103 hours.

In Experiments II and III, in which the infective dose was 28,000,000 and 8,000,000 organisms respectively (100,000 and 30,000 M.L.D.), 4 inhalational treatments per day for 4 days resulted in a 70% survival, with no survivals among the controls.

In Experiment IV a comparison was again made between parenteral and inhalational therapy following the intrabronchial inoculation of 5,000,000 organisms (17,000 M.L.D.). The 5 rats receiving 16 intramuscular injections of 500 μg each (total of 8,000 μg) over a 4-day period showed a 100% survival. Ten rats receiving 16 inhalational treatments with an estimated 2,200 μg per treatment (total of 35,000 μg), also showed a 100% survival. Of the 10 untreated control rats, 2 survived. The 2 surviving controls showed, when sacrificed at 14 days, lung lesions consisting, in one case, of an abscess containing viable Friedländer's bacilli; and in the other rat consolidation of the right lower lobe, with adhesions; whereas all treated rats, when sacrificed at 14 days, showed no lesions of any kind.

It is apparent from these experiments that the intramuscular route of administration of streptomycin was more effective in controlling an *overwhelming* dosage of Friedländer's bacilli (100,000 M.L.D.) than was the inhalational route. Moreover, that a 100% survival rate following inhalational treatment was possible only when the infective dosage of organisms was decreased to 5,000,000 (17,000 M.L.D.). And even at this dosage a smaller amount of the drug gave protection when administered intramuscularly than when it was given by the inhalational route (8,000 μg and 35,000 μg respectively), although the actual inhalational dose was probably less than half the estimated one.[1]

A possible explanation for the greater effectiveness of the intramuscular mode of administration, especially under conditions of tremendous infective dosages, can be found in the circumstance that a septicemia soon takes place, as is well known to occur with any type of Friedländer infection in rats or mice, and that this septicemia naturally is controlled more quickly by parenteral administration than by inhalational. This view receives further support from our observations[5] that streptomycin in contrast to penicillin, is poorly absorbed from the lung into the blood stream.

Summary. Streptomycin, administered to white rats by intramuscular injection or by inhalation, *following* intrabronchial inoculation of mucin-suspended Friedländer's bacilli, is highly effective in preventing a consequent pneumonia. Of 10 rats infected with 100,000 lethal doses and treated by intramuscular injection of 1,000 μg of streptomycin 4 times a day for 3 days, all survived, while 10 control, untreated rats died within 2 days. Similarly, injections of 500 μg 4 times a day for 4 days saved all of 5 rats inoculated with 17,000 lethal doses, while 8 of 10 control rats died within 4 days.

When the drug was given at similar intervals by inhalation of streptomycin aerosol to 30 rats infected intrabronchially with from 30,000 to 100,000 lethal doses, 60 to 70% of the treated animals survived following estimated dosages of from 1,000 to 1,600 μg per treatment. All of the 30 untreated control rats died. To the foregoing data it may be added that, when the dosage was reduced to 5,000,000 organisms, which constituted a dose of 17,000 M.L.D., and inhalational treatment was given at the rate of an estimated 2,200 μg per 15-minute treatment 4 times a day for 4 days, 100% of 10 treated rats survived, while 80% of 10 controls died within 4 days.

Streptomycin (sulfate) used in this study was generously supplied by Chas. Pfizer and Company, Brooklyn, N. Y.

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[5] Laurent, A. M., McIlroy, A. P., and Hadley, F. P., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 213.