

Penicillin and Streptomycin in Lungs and Blood Serum of Rats Following Inhalational and Intramuscular Administration.*

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(Introduced by Philip Hadley.)

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In connection with a study of the therapeutic effects of penicillin and streptomycin aerosols on experimental pneumococcus and Friedländer's pneumonia in rats,^{1,2} it became of interest to ascertain the concentration of these drugs in lungs and blood serum of normal rats at various times following administration by the inhalational and intramuscular routes.

Little work has been reported on the detection of these drugs in the tissues of animals. Bryson, Sansome and Laskin³ recovered a "bacteriostatic substance" from the lungs and urine of a rabbit exposed to penicillin aerosol for 2 hours; also from the lungs of 4 mice exposed for 1 hour. Kornegay, Forgacs and Henley⁴ reported that in guinea pigs streptomycin was absent in lung, spleen and liver extracts following parenteral administration of this drug.

Blood levels of these drugs have been carefully studied by many workers, particularly following parenteral administration. Zintel and co-workers⁵ have shown that streptomycin is detectable in the blood for 6 hours following a single intravenous injection as compared with 2½ to 3 hours in the case of penicillin. Wilson, Hammond, Byrne and

Bliss⁶ found that rats given penicillin by the inhalational route showed a higher blood level within a few minutes than those animals receiving the drug by the intramuscular or oral route; also, that the drug was maintained longer in the blood following inhalation.

Methods. Drug Dosage. Ten normal, albino rats weighing approximately 250 g were chosen for each experimental group. In the *penicillin* experiments those receiving the drug parenterally were given a single intramuscular injection of either 2,000 or 5,000 units of calcium penicillin (Pfizer) in 0.85% salt solution. The rats receiving penicillin by inhalation were exposed for 15 minutes to nebulized penicillin produced from a saline solution containing 40,000 units per cc. Approximately 2,000 units were estimated to have been inhaled by each rat during this exposure, but the *actual* retained inhalational dose was probably much less. The technic used in administering the aerosol and the method of calculating the inhalational dose were described in a previous report.¹

In the *streptomycin* experiments intramuscular injections of either 2,500 or 5,000 µg of streptomycin sulfate (Pfizer) were given. Rats receiving the drug by inhalation were exposed for 15 minutes to a mist produced by nebulizing a solution of streptomycin containing 75,000 µg per cc of salt solution. The average *estimated* inhalational dose per rat was approximately 2,300 µg.

Preparation of Lung Extract. In each ex-

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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 Hadley, F. P., Laurent, A. M., and Onslow, J. M., *Proc. Soc. Exp. Biol. and Med.*, 1948, **68**, 210.

³ Bryson, V., Sansome, E., and Laskin, S., *Science*, 1944, **100**, 33.

⁴ Kornegay, G. B., Forgacs, J., and Henley, T. F., *J. Lab. and Clin. Med.*, 1946, **31**, 523.

⁵ Zintel, H. A., Flippin, H. F., Nichols, A. C., Wiley, M. M., and Rhoads, J. E., *Am. J. Med. Sc.*, 1945, **210**, 421.

⁶ Wilson, C. E., Hammond, C. W., Byrne, A. F., and Bliss, E. A., *Bull. Johns Hopkins Hosp.*, 1946, **79**, 451.

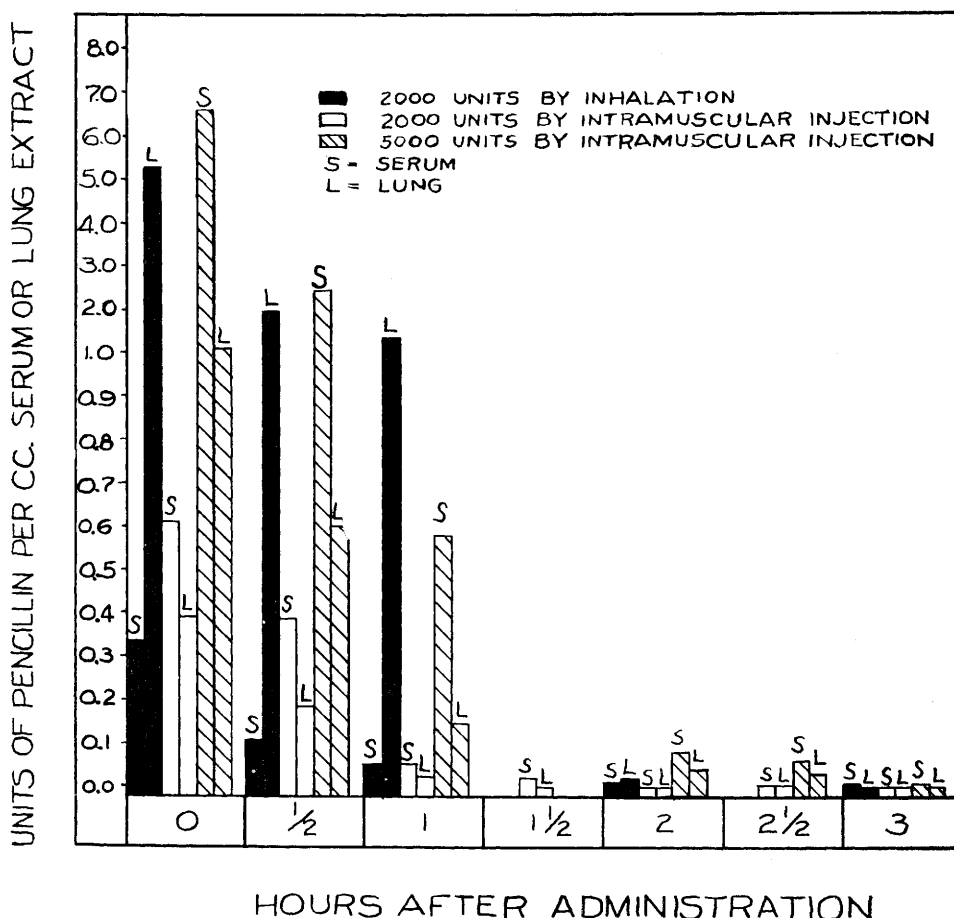


Fig. 1.
Penicillin levels in rat serum and lungs.

periment 2 rats were sacrificed at times ranging from a few minutes to 3 hours after administration of the drugs. Under ether anesthesia each rat was exsanguinated by the removal aseptically of 6 to 9 cc of blood from the heart, followed by the severance of blood vessels in the neck to permit further bleeding. The lungs were removed aseptically *in toto*, and each lobe, dissected away from the trachea, was blotted on sterile filter paper to remove traces of blood adhering to the surface. They were then ground with sand and 1.5 cc of salt solution and the resulting paste transferred to tubes and centrifuged for 5 minutes at about 5,000 R.P.M. The supernatant material was removed and stored in the ice-box, together with the blood serum, until drug

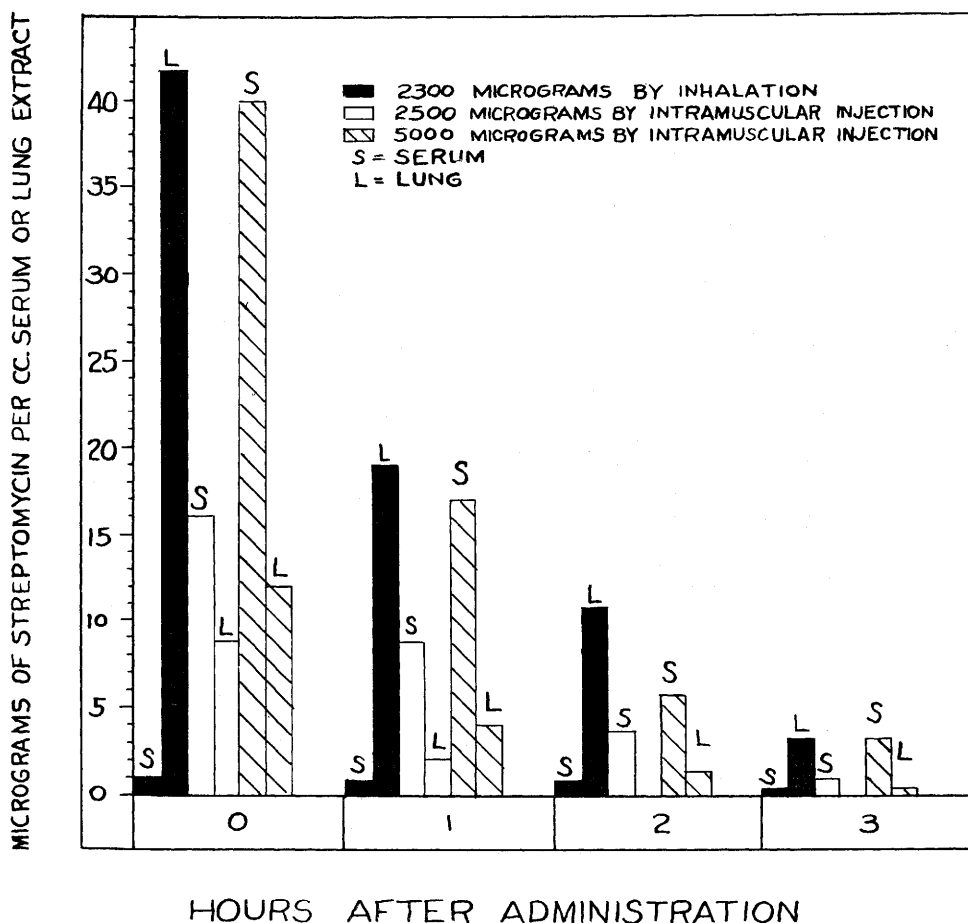
concentrations were determined on the following day. Separate determinations were made for each animal.

Methods of Assay. Penicillin in lung extracts and serums was determined by the method of Rammelkamp⁷ using a 16 hour broth culture of the C203M strain of hemolytic streptococcus. Veal-infusion broth (pH 7.4) was used to dilute samples and penicillin standard.

Streptomycin was determined by the method of Donovanick and Rake⁸ with some modifications. A 6-hour broth culture of

⁷ Rammelkamp, C. H., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 95.

⁸ Donovanick, R., Hamre, D., Kavanagh, F., and Rake, G., *J. Bact.*, 1945, **50**, 623.



HOURS AFTER ADMINISTRATION

Fig. 2.

Streptomycin levels in rat serum and lungs.

Friedländer's bacillus (No. 837) was the test organism, and Difco yeast broth (pH 7.0) was used to dilute the streptomycin standard and samples.

Results. Penicillin Concentrations. Fig. 1 presents the summarized data for several experiments. The solid bars, representing units of penicillin per cc of serum or lung extract following inhalation, show the average concentration for 10 rats after each time interval. The striped and open bars, representing concentrations following intramuscular injection, show average figures for 2 rats after each time interval.

Lungs. It may be seen from the graph that the lungs contained a much higher concentration of penicillin (5.3 units/cc lung extract) immediately (or within the first few minutes)

following a 2,000 unit inhalational dose than following either 2,000 or 5,000 units by injection (0.39 and 1.17 units/cc lung extract, respectively). This was true also at one-half and 1 hour intervals. It is noteworthy that the high lung concentration following 2,000 units by inhalation persisted for at least 1 hour, at which time it was 60 times greater than that resulting from a 2,000 unit intramuscular dose, and 10 times greater than that following a 5,000 unit intramuscular dose. At 2 hours penicillin was still detectable in the lungs following inhalation, but it was absent at this time following a similar intramuscular dose. At 3 hours it was absent in all lung samples.

Serum. Comparing the serum concentrations following the 3 methods of administra-

tion it may be seen that a 2,000 unit inhalational dose resulted immediately in an effective level (0.33 units/cc) which dropped to 0.04 units/cc at 1 hour. A 2,000 unit intramuscular dose resulted in a serum level twice as high immediately (0.6 units/cc) but at 1 hour it equaled the serum concentration attained at this period following inhalation. The 5,000 unit intramuscular dose gave the highest serum levels which were maintained at inhibitory concentrations through 2½ hours.

Streptomycin Concentrations. In Fig. 2 are presented figures for streptomycin levels in serum and lungs at various times following administration of the following doses: 2,300 µg by inhalation, 2,500 µg and 5,000 µg by intramuscular injection. Each bar in the diagram represents the average value obtained from determinations made on 2 rats (in 70% of the assays the values were identical for the 2 animals).

Lungs. Immediately following inhalation of 2,300 µg of streptomycin the concentration in the lungs was 41.6 µg/cc of lung extract, whereas intramuscular injections of 2,500 and 5,000 µg resulted in immediate concentrations of only 8.7 and 11.6 µg, respectively. At 1 hour following inhalation the lung concentration was 18.7 µg, while only 2 and 4 µg, respectively, were detected following injection of 2,500 and 5,000 µg. Even at 2 and 3 hours after inhalation the lungs contained 10 µg and 3 µg, respectively, although none was detectable at these times following a similar dose by injection. At 2 and 3 hours following

a 5,000 µg injection 1.2 and 0.4 µg, respectively, were found.

Serum. It is to be noted that the concentration of streptomycin in the serum following the 2,300 µg inhalational dose remained at less than 1 µg/cc at all 4 time intervals. This indicates that, in spite of the high lung concentration, this drug is poorly absorbed into the blood from the lungs. A 2,500 µg injection resulted in serum levels ranging from 15 µg/cc immediately to 1 µg at 3 hours. And following a 5,000 µg injection serum levels ranged from 40 µg/cc immediately to 3 µg at 3 hours.

Summary. 1. Penicillin and streptomycin were found in the lungs of rats in far greater concentrations following inhalational administration than after intramuscular injection of similar and even higher doses.

2. Penicillin was retained in the lungs in a high concentration for 1 hour following inhalation. It was still detectable in smaller amounts at 2 hours.

3. Streptomycin was retained in the lungs in an effective concentration for at least 3 hours following inhalation.

4. Penicillin was present in the blood stream in effective concentrations for 2 hours following inhalation.

5. Streptomycin was poorly absorbed from the lungs into the blood following inhalation.

Penicillin and streptomycin used in this study were generously supplied by Chas. Pfizer and Company, Brooklyn, N. Y.

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Effect of Infused Streptomycin in the Mammary Gland.*

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With the establishment of streptomycin as a potent chemo-therapeutic agent in the treat-

ment of many infections resistant to either sulfonamides or penicillin the possibility of its use in the treatment of some forms of infectious bovine mastitis became apparent. This present study was undertaken to deter-

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