Streptomycin in EXPERIMENTAL PLAGUE

STUART F. QUAN, LUCILE E. FOSTER, ADELIEN LARSON, AND KENNETH F. MEYER
From the George Williams Hooper Foundation, University of California, San Francisco, California.

In July, 1944, Dr. Selman A. Waksman invited a study of therapeutic properties of streptomycin in *Pasteurella pestis* infections. Preliminary tests with a one gram sample of the crude antibiotic gave very promising results. Subsequently, larger lots of streptomycin were obtained through generosity of Dr. I. M. Carlisle, Merck and Co., Inc., Rahway, N. J. A confidential report of results was made in 1945 to the committee on Medical Research of the Office of Scientific Research and Development. Publication times for that report and later studies are indefinite, but the more significant results are summarized here.

(1) RESULTS OBTAINED IN VITRO. Streptomycin in hormone broth (1,250 μg/cc) kills 100 million virulent *P. pestis* (Shasta) in 15 minutes. The same number of organisms are destroyed in 4, 12, 24, 48, and 120 hours, respectively, by 313 μg/cc, 78 μg/cc, 39 μg/cc, 29 μg/cc, and 5 μg/cc. One hundred thousand organisms of a recently isolated human strain (Modoc) were killed by 0.2 μg/cc, and an equal amount of the "Shasta" strain by 1.9 μg/cc in 72 hours. Amounts varying from 0.4 to 4.0 μg/cc proved bactericidal to six plague strains of Hawaiian, Egyptian, Indian, and Californian origin in five days. From one to 16 units/cc of dihydrostreptomycin are required to sterilize these strains in five days. Avirulent strains are more resistant: for example, the E. V. 76 plague bacillus (Girard)[1] is killed in the presence of 16 units/cc/five days, strains 14 and 1122 (Jawetz and Meyer)[2] by eight units/cc and the Tjiwidej strain (Otten)[3] by four units/cc. In the presence of 10% blood serum, 40 units/cc were required to kill 10,000 virulent *P. pestis* "Yreka" in 48 hours. Other factors, such as chemical composition of pH of the suspending medium, concentration of antibiotic, and age and density of culture, influence bactericidal activity of streptomycin on *P. pestis*. Five thousand μg/cc in broth destroyed 22,000 million virulent *P. pestis* (Yreka)/cc, while the same concentration sterilized 33,000 million/cc in physiologic saline. Four virulent strains trained to resist streptomycin in the amount of 5,000 μg/cc were avirulent and resisted only 2,500 units/cc dihydrostreptomycin.

(2) EFFECT ON MICE AND GUINEA PIGS INFECTED SUBCUTANEOUSLY. Relative sensitivity of *P. pestis* to streptomycin per-

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[1] Girard, *Bull. office int. d'hyg. publ.*, 1936, 28, 1078.
[2] Jawetz and Meyer, *J. Infect. Dis.*, 1943, 73, 124.
[3] Otten, *Indian J. Med. Res.*, 1936, 24, 73.

mits effective therapy of experimental bubonic disease caused by 100 to 1,000 multiples of the M.L.D. in highly susceptible (ABC) mice. Treatment usually begins on the 48th hour after subcutaneous introduction of the bacilli, when infection is generalized, a bacteremia is well established in 40 to 60% of the animals, and the immunity mechanism is partially damaged by toxins. Irrespective of dosage or frequency of administration, sulfonamides save an average of only 35% of mice at this stage of infection. In 70 separate experiments, treating as many groups of 20-50 mice with varying amounts of streptomycin, advanced experimental bubonic plague was completely cured with 500 μg/three hours for three days, or a total of 12,000 μg (12.0 mg.). The median effective dose was 1,000 to 1,250 μg for a 20 g mouse, or 50 to 62 μg/g when one intraperitoneal injection was given on the 48th hour of infection. Bacteriological autopsies demonstrated that on the 14th hour after injection of the antibiotic, the spleens of four mice sacrificed were sterile; however, plague bacilli could be cultured from the heart blood of 2/4, the livers of 3/4, and the lymph nodes of 4/4. Organs of control mice showed heavy growth of *P. pestis* in cultures and microcopically. Begun on the 48th hour of infection, 100 μg of streptomycin injected at six-hour intervals for 192 hours, or 32 injections in all (a total of 3.2 mg of streptomycin per mouse), always sterilize the blood stream, spleen and liver; but since the infection persists in lymph nodes, about 40% of the animals ultimately succumb. The rate of survival in mice is directly proportional to the amount of streptomycin administered. A large dose of antibiotic at the outset of infection probably kills or injures the bacillus, inhibits its multiplication and enhances receptivity to phagocytosis, which effects its removal from the circulation. There is a possibility that the regional bubo, with its abundant necrotic tissue and large number of plague bacilli, is not immediately affected, and thus serves as a seedbed for relapses and a continuous source of toxin when the immunity mechanism is not completely mobilized. The larger the dose, the greater the possibility for streptomycin to diffuse into necrotic areas, and the shorter the course of therapy needed. In the mouse the greatest dose, or that determined by toxicity, is more than ten times the effective dose that was used in the treatment of plague infections.

Bubonic plague infections which have progressed to an extent comparable to 48-hour infections in mice occur in guinea pigs 120 hours after subcutaneous injections of 1,000 multiples of the M.L.D. With a streptomycin dose of 20,000 μg/kg or approximately 10 mg per day, a total of 100 mg over a period of 10 days, 80 to 100% of the 40 guinea pigs used in experiments were cured. It requires less streptomycin to cure guinea pigs in a stage of mild septicemia than to cure mice, on the basis of units of antibiotic to grams of body weight. Administration of 25,000 to 50,000 μg/kg in three doses, or a daily injection of 37,500 to 75,000 μg/500 g guinea pig of early streptomycin preparations proved toxic, but recent lots of the antibiotic in the same doses are well tolerated.

In the tests under consideration, and those of Wayson and McMahon[4], persistence of plague bacilli in regional buboes despite large doses of the antibiotic suggested local chemotherapy. Guinea pigs with well-developed buboes on the 120th hour after subcutaneous administration of 100,000 *P. pestis*, were injected around the local swelling with 12,500 μg in 0.5 cc of physiologic saline at 12-hour intervals for 10 days. All were cured and plague lesions were sterilized. Sodium sulfamerazine administered in the same manner in the amount of 500 mg/kg/12 hours was inhibitory but not bactericidal, and cured no more than 40% of infected animals. Local injections of streptomycin proved very irritating, and even though buboes became rapidly sterile, necrotic areas were much larger than

[4] Wayson and McMahon, *J. Lab. and Clin. Med.*, 1946, 31, 323.

DYNAMICS OF STREPTOMYCIN ON EXPERIMENTAL SEPTICEMIC PLAGUE IN MICE
(INTRAPERITONEAL INFECTION) EXPERIMENT 97

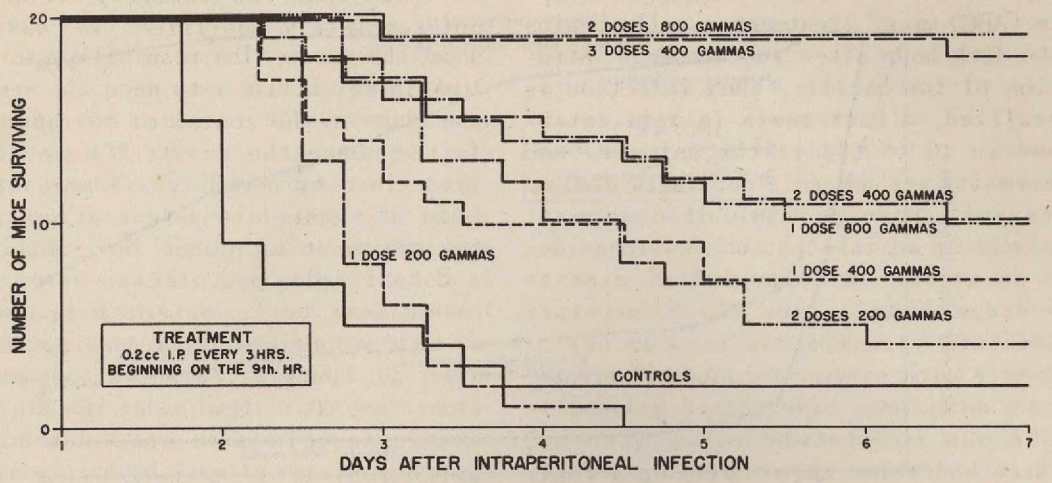

FIGURE I.

STREPTOMYCIN IN THE THERAPY OF THE 36-HOUR INTRANASAL PLAGUE

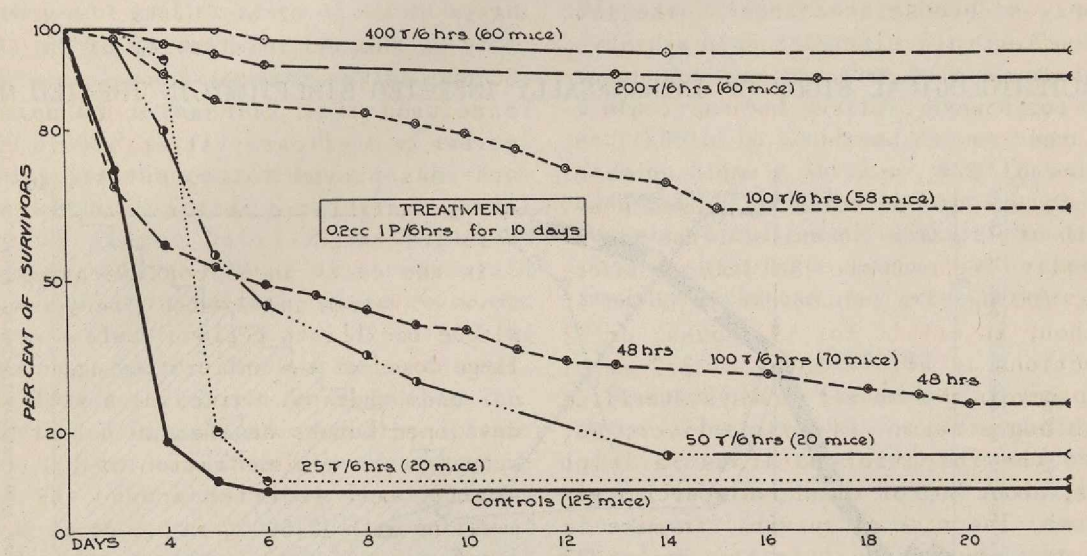

FIGURE II

those of controls or of animals treated systemically.

(3) EFFECT ON SEPTICEMIC PLAGUE IN MICE. To establish maximum effectiveness of streptomycin, a rapidly progressive SEPTIMIC infection was produced in slightly-resistant Swiss mice by intraperitoneally injecting 2,000 *P. pestis*. Bacteremia and toxemia exist in such mice by the 9th-12th hour after infection, and death takes place in 34 to 72 hours. In some respects, this infection model resembles the clinical form of human septicemic plague induced by direct blood stream infection through several flea bites. Repeated tests of the kind shown in Fig. 1 conclusively demon-

INTRANASAL PLAGUE TREATED WITH SERUM AND SULFADIAZINE

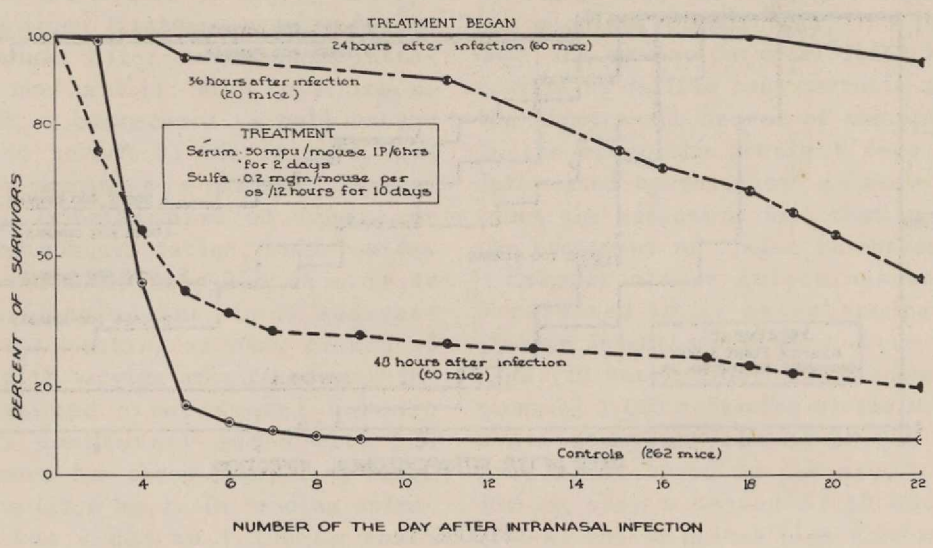

FIGURE III

BACTERIOLOGICAL STUDY OF INTRANASALLY-INFECTED STREPTOMYCIN TREATED MICE

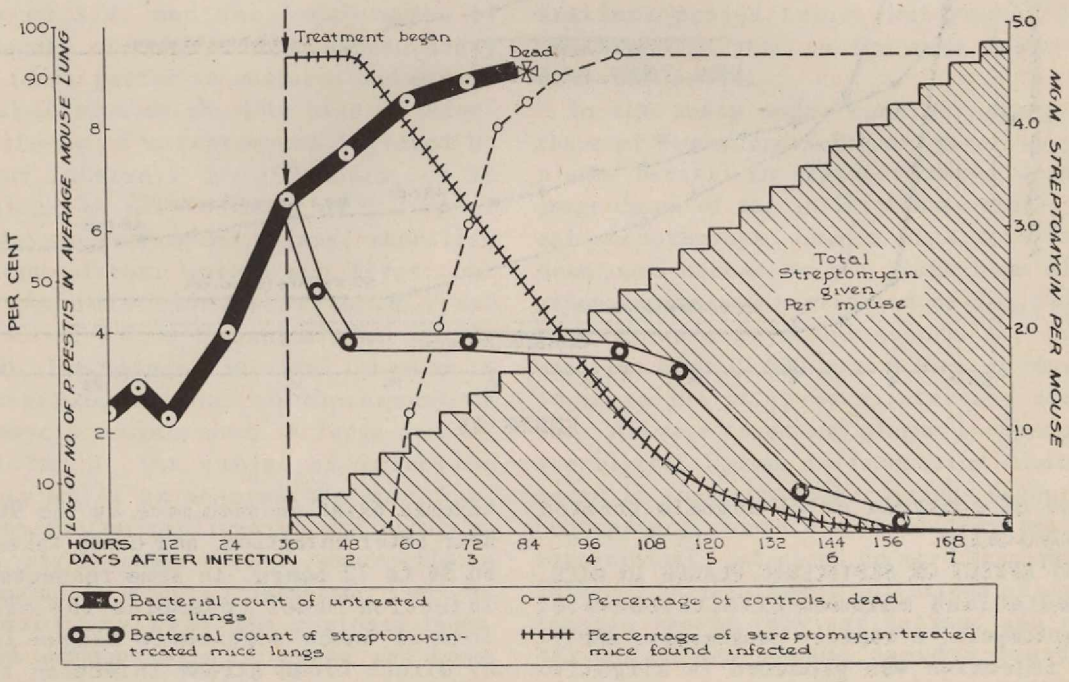

FIGURE IV.

strated that two to three large doses of 400 to 800 μg at three-hour intervals, or a total of 1,200 to 1,600 μg, cured 80 to 90% of non-bubonic septicemic plague infections. Small doses merely delay death. If treatment is postponed until the 18th or 24th hour, two doses of 800 μg each cure only 25 to 45% of infected mice. Only early treatment with large doses of streptomycin cures septicemic plague in mice. Sulfadiazine and highly potent antiplague serum proved ineffective under identical experimental conditions. There is at present no evidence that sulfonamides synergistically enhance the remedial action of streptomycin. On the other hand, numerous experiments furnished data that potent anti-plague serum administered simultaneously with small doses of streptomycin increased the percentage of cures. Finally, active immunization with plague antigens potentiates the action of streptomycin both in mice and in guinea pigs and the partial immunity so gained allows use of smaller doses of streptomycin.

(4) EFFECT OF PNEUMONIC PLAGUE IN MICE. Pneumonic plague infections may be produced in mice by intranasal instillation of 2,500-5,000 *P. pestis* in 0.05 cc of saline; lobular lesions recognizable on the 36th hour after infection contain several million plague bacilli. As might be expected, 200 to 400 μg of streptomycin hydrochloride or sulfate, given every six hours, effectively cure 90 to 95% of infections. Smaller doses or delayed treatment reduces the chance for cures (Fig. 2). Sulfadiazine in combination with antiplague serum is less effective than streptomycin (Fig. 3). The remarkable bactericidal action of streptomycin is fully documented by periodic bacteriological autopsies of treated and untreated mice (Fig. 4). Six hours after treatment with 200 μg had begun, the number of plague bacilli found in the lungs of treated mice was reduced to approximately 60,000, while in untreated animals it had advanced to 10,000,000. By the 12th to 24th hours of treatment, the spleen became sterile and 5000 or less organisms were counted in the lungs. By the 96th hour after infection, when all untreated mice had died, the lungs and bronchial lymph nodes of treated mice were either sterile or contained only a few thousand plague bacilli in the abscess-like patches of pneumonia. No plague bacilli have been isolated from lungs or lymph nodes 100 hours after treatment with streptomycin. These results fully attest to the remarkable therapeutic efficacy of a total of five mg of streptomycin in experimental plague of mice. They justify an exception that it will be equally effective in human pneumonic plague if administered early and in adequate dosage.

(5) SUGGESTED SCHEDULE OF TREATMENT IN HUMAN PLAGUE. Streptomycin is the most effective therapeutic agent thus far discovered for the treatment of bubonic, septicemic and pneumonic experimental plague infections in mice and guinea pigs. It is recommended that human plague be treated as soon as diagnosed with daily doses of two g of streptomycin in bubonic plague, and four to six g in the septicemic and pneumonic diseases; injections should be given at four-six hour intervals for the first two days. The dose may then be reduced, but in order to prevent clinical recurrences treatment should be continued for at least eight days on a one g level or substituted with adequate sulfadiazine therapy. In profound toxemia, simultaneous administration of a potent antiplague serum, to assist the immunity mechanism, may prove beneficial.

SUMMARY

Streptomycin in the amounts of 0.4 to 4.0 μg/cc is bactericidal for different strains of *P. pestis* in five days. Advanced experimental bubonic plague in mice is completely cured with 500 μg/three hours for three days, or a total of 120 mg. Between 80 to 90% of the mice in a state of septicemic plague may be saved with a total of 1,200 to 1,600 μg. The remarkable bactericidal action of streptomycin is best demonstrated on experimental pneumonic plague; five mg of the anti-biotic sterilize lungs and lymph nodes within 100 hours after treatment has been instituted. It is recommended that human plague be treated as soon as diagnosed with two to four g of streptomycin daily depending on the state of infection.