

are therapeutically effective in the treatment of experimental tuberculosis of guinea pigs. Both are more effective in suppressing tuberculous infection by the institution of treatment within a few days after inoculation with virulent tubercle bacilli than in the treatment of established lesions developing 2

weeks or longer after infection has been established. Particularly noteworthy therapeutic results were observed following combined streptomycin and diasone therapy, suggesting that these 2 compounds exert a synergistic or additive therapeutic effect in the treatment of experimental tuberculosis.

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Acquired Resistance of *Treponema pallidum* to Penicillin.

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Various pathogenic penicillin-susceptible bacteria have been found to apparently acquire resistance to penicillin both *in vitro* and *in vivo*. The evidence, however, is not conclusive since so-called acquired resistance may be nothing more than the survival of variants in cultures of any bacterium possessing a higher natural resistance than usual when exposed to the compound *in vitro* or *in vivo*.

Be that as it may, the question of whether or not *T. pallidum* may develop so-called acquired resistance or tolerance to the compound is one of great practical importance and especially in relation to the treatment of chronic syphilis. In this connection Tung and Frazier¹ have reported that cultures of the Reiter strain of alleged *T. pallidum* did not develop any evidences of increased resistance or tolerance to penicillin after being subjected to 15 passages through media containing the compound although Dunham and his associates² have reported that the inadequate treatment of a syphilitic rabbit resulted in acquired resistance of *T. pallidum* to penicillin *in vitro*.

In our experiments 4 rabbits (first series) were inoculated intratesticularly with the Nichols-Hough strain of *T. pallidum*. About

5 weeks later all showed acute syphilitic orchitis with strongly positive dark-field examinations. Two rabbits were then given 500 units and 2 1000 units of penicillin* by intramuscular injection in sterile saline solution at 9 a.m., 1 p.m. and 4 p.m. daily for 9 doses, totalling 4500 and 9000 units respectively per kg of weight, over a period of 3 days. All showed clinical improvement with negative dark-field examinations in from 7 to 14 days after the institution of treatment but all thereafter relapsed clinically with positive dark-field examinations.

At the end of 3 weeks 4 rabbits (second series) were inoculated intratesticularly with testicular material obtained from the testicles of the 4 treated animals of the first series. Acute syphilitic orchitis with strongly positive dark-field examinations developed in 5 to 6 weeks at which time the animals received the same treatment as described above. All showed marked clinical improvement with negative dark-field examinations about 7 days after the institution of treatment which persisted over a period of about 2 weeks when 3 of the treated animals relapsed clinically with positive dark-field examinations. One rabbit, however, receiving a total of 9000 units per kg remained clinically and dark-field negative over a period of 6 weeks but lymph node transfers to fresh animals at

¹ Tung, T., and Frazier, C. N., *Am. J. Syph., Gon. and Ven. Dis.*, 1946, **30**, 205.

² Dunham, W. B., Hambre, D. M., McKee, C. M., and Rake, G., *Proc. Soc. Exp. Biol. and Med.*, 1944, **55**, 158.

* Commercial penicillin (lot No. 45072102) kindly supplied by the Commercial Solvents Corp., New York, N.Y.

that time gave positive results, indicative of incomplete "cure."

At the end of 3 weeks 4 rabbits (third series) were inoculated intratesticularly with testicular material obtained from 3 of the animals of the second series. All developed acute syphilitic orchitis with strongly positive dark-field examinations about 5 weeks later at which time they received the same treatment as previously employed. All animals showed healing with negative dark-field examinations about 7 days after the institution of treatment. Three rabbits, however, relapsed clinically with positive dark-field examinations about 5 weeks after the institution of treatment; the fourth animal receiving a total of 9000 units per kg remained clinically well, with negative dark-field examinations, up to 6 weeks at which time lymph node transfers were made with positive results.

At the end of 3 weeks 4 rabbits (fourth series) were inoculated intratesticularly with testicular material obtained from 3 of the animals of the third series. All developed acute syphilitic orchitis with strongly positive dark-field examinations about 5 weeks later at which time they received the same treatment as previously employed. All animals showed healing with negative dark-field examinations about 6 days after the institution of treatment. Two animals receiving a total of 4500 units per kg relapsed clinically with positive dark-field examinations about 4 weeks after the institution of treatment. The 2 animals receiving a total of 9000 units per kg, however, remained clinically well with negative dark-field examinations and at the end of 8 weeks lymph node transfers were made; one was positive and the second negative over a period of 9 weeks, indicative of a complete cure.

At the end of 3 weeks 8 rabbits (fifth series) were inoculated intratesticularly with testicular material obtained from the 2 rabbits of the fourth series with relapsing infections. Acute syphilitic orchitis developed about 5 weeks later with strongly positive dark-field examinations at which time treatment was instituted. Two rabbits were given intramuscular injections of 100 units per kg

at 9 a.m., 1 p.m. and 4 p.m. for 10 doses (totalling 1000 units); both showed clinical improvement with negative dark-field examinations but relapsed at the end of about 10 days. Two rabbits were given a total of 5000 units per kg with temporary negative dark-field examinations but likewise relapsed. Two rabbits were given a total of 10,000 units per kg; both became dark-field negative in about 72 hours which persisted over a period of 6 weeks. At the end of that time lymph node transfers were made and the animals kept under observation for 3 months; one was positive and the second negative, indicative of complete biologic cure. Two rabbits were given a total of 50,000 units per kg; both became dark-field negative in about 72 hours which persisted over a period of 8 weeks. At the end of that time lymph node transfers were made and the animals kept under observation for 3 months; both were negative and indicative of complete biologic cure.

At the same time 8 additional rabbits were inoculated intratesticularly with the original or parent strain of *T. pallidum* as controls. Acute testicular syphilis developed in all in about 5 weeks with strongly positive dark-field examinations at which time treatment was instituted. The latter was exactly as described above, 2 rabbits receiving a total of 1000 units, 2 a total of 5000 units, 2 a total of 10,000 units and 2 a total of 50,000 units per kg. Practically identical results were observed.

Summary. The minimal curative dose of a commercial penicillin in aqueous solution by intramuscular injection 3 times a day for 10 doses in succession was apparently slightly more than 1000 units per kg (totalling 10,000 units) and probably somewhat less than 5000 units per dose (totalling 50,000 units) in the treatment of acute testicular syphilis of rabbits inoculated with the Nichols-Hough strain of *T. pallidum*. This strain did not show any evidence of acquired resistance or tolerance to penicillin after 3 consecutive passages through the testicles of rabbits treated with subcurative amounts of the compound.