

Streptomycin and Diasone in the Treatment of Experimental Tuberculosis of Guinea Pigs.

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Streptomycin has yielded encouraging results in the treatment of experimental tuberculosis of guinea pigs¹⁻³ and of Swiss mice,⁴ especially by exerting a suppressive effect upon the development of lesions when administered within a few days to 2 weeks after inoculation with virulent tubercle bacilli. Varying dosage has been employed but daily total doses of 5000 to 6000 units in saline solution per guinea pig (approximately 10,000 to 15,000 units per kg), divided into 4 to 6 subcutaneous or intramuscular injections, have been found satisfactory and generally well tolerated. Various derivatives of diamino-diphenylsulfone have also been found somewhat effective in the treatment of experimental tuberculosis of these animals.^{5,6}

The purpose of our experiments was to determine not only the therapeutic effectiveness of streptomycin in experimental tuberculosis of guinea pigs, but the possible synergistic or additive effects of combined streptomycin and diasone (a diamino-diphenylsulfone derivative) therapy in this disease.

In the first experiment 20 guinea pigs varying in weight from 320 to 485 g were inoculated subcutaneously with 0.5 mg of culture H42733 of human tubercle bacilli per 100 g body weight*. Ten were kept as untreated controls and 10 subjected to strep-

tomycin treatment beginning on the 3rd day after inoculation.[†] The dose of the compound per animal was 1500 units in saline solution, by subcutaneous injection, at 9:30 a.m. and 1:30 p.m. with an intramuscular injection of 6000 units suspended in sterile peanut oil and 3% beeswax at 4:30 p.m., totalling 9000 units per animal per day. After 10 to 14 days of treatment, however, many of the animals showed evidences of toxic effects of the compound and there were 2 deaths. Under the circumstances treatment was interrupted for 4 days and then resumed with a total daily dose of 5000 units which was raised to 6000 units beginning with the 4th week of treatment and continued for a total of 72 days after inoculation; all surviving treated and untreated animals were sacrificed for autopsy examinations. Microscopic and macroscopic examinations of the spleen, liver and lungs were made of animals succumbing during the experiment or sacrificed at its termination. Assays of the serum in a number of animals showed about 6.5 units of streptomycin per cc 20 minutes after the subcutaneous injection of 1500 units in saline solution, which dropped to about 2.5 units per cc at the end of 1 hour and to between 1-2 units at the end of 2 hours. Two hours after intramuscular injections of 3000 units in peanut oil and 3% beeswax no streptomycin could be detected in the serum with 1 to 2 units per cc at the end of 4 hours and none at the end of 6 and 18 hours. It would appear, therefore, that streptomycin suspended in peanut oil and beeswax is not absorbed as readily and uniformly from the muscles of guinea pigs as penicillin. Similar results have been observed in human adults following single intramuscular injections of 250,000 and 500,000 units of streptomycin

¹ Feldman, W. H., and Hinshaw, H. C., *Proc. Staff Meet. Mayo Clinic*, 1944, **19**, 594.

² Feldman, W. H., Hinshaw, H. C., and Mann, F. C., *Am. Rev. Tuberc.*, 1945, **52**, 269.

³ Smith, M. I., and McCloskey, W. T., *Pub. Health Rep.*, 1945, **60**, 1129.

⁴ Youmans, G. P., and McCarter, J. D., *Am. Rev. Tuberc.*, 1945, **52**, 432.

⁵ Feldman, W. H., Hinshaw, H. C., and Moses, H. E., *Am. J. Med. Sc.*, 1944, **207**, 290; *Arch. Path.*, 1944, **36**, 64.

⁶ Callomon, F. T., and Raiziss, G. W., *Am. Rev. Tuberc.*, 1946, **53**, 374.

* Kindly supplied by the Phipps Institute, Philadelphia, Pa.

[†] Streptomycin sulfate kindly supplied by the Abbott Laboratories, North Chicago, Ill.

TABLE I.
Streptomycin in Treatment of Experimental Tuberculosis of Guinea Pigs.

	No.	Duration of life (days after inoculation)	Results of autopsies*		
			Spleen	Liver	Lungs
Treated	1	Killed 72 days	+	—	+
	2	" " "	+	—	++
	3	" " "	+	—	++
	4	" " "	—	—	+
	5	" " "	+++	—	++++
	6	Died 19 "	—	++	++++
	7	Killed 72 "	+	+	++++
	8	Died 15 "	++	—	—
	9	Killed 72 "	++	+	++
	10	Died 37 "	++	+	++++
Controls	11	Died 56 "	++	+++	++++
	12	Killed 72 "	++	—	++++
	13	" " "	+++	—	++++
	14	" " "	+++	—	+++
	15	Died 25 "	—	++	—†
	16	Killed 72 "	++	++	++++
	17	" " "	++	++	++++
	18	" " "	+++	—	+++
	19	" " "	+	+++	++++
	20	" " "	+++	+	++++

* — = no lesions; + = occasional small tubercles or one small solitary tubercle; ++ = many microscopic tubercles; +++ = moderate involvement showing focal rather than diffuse tuberculosis with or without necrosis; ++++ = severe progressive tuberculosis with necrosis.

† Acute nontuberculous pneumonia.

suspended in peanut oil and 4% beeswax.⁷

The results are shown in Table I and may be summarized as follows: (a) of 10 untreated controls 2 succumbed; (b) of 10 treated animals 3 succumbed although 2 of these deaths (Nos. 6 and 8) were thought to be due to the toxicity of streptomycin in the high initial dosage of 9000 units per animal per day; (c) all of the treated animals presented tuberculous lesions at autopsy but their incidence and severity were somewhat less than observed among the untreated controls and indicative of some suppressive therapeutic activity on the part of the streptomycin in the doses and method of administration employed.

In the second experiment 32 guinea pigs, weighing 350 to 500 g, were inoculated subcutaneously with 0.75 mg of the same culture per 100 g of weight in order to produce more severe infections, and treatment was not instituted until 18 days after inoculation. One group of 8 animals was then given 1500

units of streptomycin in saline solution by subcutaneous injection at 9:30 a.m. and 1:30 p.m., and 3000 units in peanut oil and 3% beeswax intramuscularly at 4:30 p.m., totalling 6000 units per day for 32 days. A second group of 8 animals were given about 210 to 240 mg of diasone[‡] containing 10% sodium bicarbonate as a stabilizer and equivalent to about 190 to 215 mg of pure diasone in food per day for 32 days. A third group of 8 animals were given a similar daily dose of diasone for 32 days, along with injections of streptomycin which were started on the 4th day of treatment, covering a period of 28 days. A 4th group of 8 animals were kept as untreated controls. All animals succumbing during the experiment were subjected to macroscopic and microscopic examinations of the spleen, liver and lungs for tuberculous lesions and all surviving the total of 50 days following inoculation were sacrificed and subjected to the same postmortem examinations.

⁷ Kolmer, J. A., Bondi, A., Jr., Warner, F. H., and Dietz, C., *Science*, 1946, **104**, 315.

[‡] Kindly supplied by the Abbott Laboratories, North Chicago, Ill.

TABLE II. Streptomycin, Diasone and Combined Streptomycin-Diasone Therapy in the Treatment of Experimental Tuberculosis of Guinea Pigs.

Treatment	No.	Duration of life (days after inoculation)	Results of autopsies*			Treatment	No.	Duration of life (days after inoculation)	Results of autopsies*		
			Spleen	Liver	Lungs				Spleen	Liver	Lungs
Streptomycin	1	Died 31 days	+	+	+	Streptomycin and Diasone	17	Killed 50 days	+	+	+
	2	" "	+	+	+		18	" "	+	+	+
	3	" 32 "	+	+	+		19	" "	+	+	+
	4	Killed 50 "	+	+	+		20	" "	+	+	+
	5	" "	+	+	+		21	" "	+	+	+
	6	" "	+	+	+		22	" "	+	+	+
	7	Died 30 "	+	+	+		23	" "	+	+	+
	8	" "	+	+	+		24	" "	+	+	+
Diasone	9	Killed 50 "	—	+	+	Controls	25	Died 43 "	+	+	+
	10	" "	+	+	+		26	Killed 50 "	+	+	+
	11	" "	+	+	+		27	" "	+	+	+
	12	Died 48 "	+	+	+		28	Died 45 "	+	+	+
	13	Killed 50 "	+	+	+		29	" 38 "	+	+	+
	14	" "	+	+	+		30	" 34 "	+	+	+
	15	Died 49 "	+	+	+		31	" 29 "	+	+	+
	16	Killed 50 "	+	+	+		32	" 27 "	+	+	+

* — = no lesions; + = occasional small tubercles or one small solitary tubercle; +++ = many microscopic tubercles; +++ = moderate involvement showing focal rather than diffuse tuberculosis with or without necrosis; +++ = severe progressive tuberculosis with necrosis.

The results are shown in Table II and may be summarized as follows: (a) 7 of the 8 untreated control animals succumbed to extensive tuberculous infection in 23 to 43 days after inoculation; (b) 5 of the 8 animals treated with streptomycin succumbed in 30 to 32 days after inoculation; (c) 2 of the 8 animals treated with diasone succumbed in 48 and 49 days respectively after inoculation and (d) all animals treated with diasone and streptomycin survived. It has been very difficult to evaluate the results in relation to the distribution and severity of tuberculous infections of the spleen, liver and lungs, but in general terms it appears that these were somewhat less severe among the animals treated with streptomycin and diasone alone and by streptomycin and diasone.

Discussion. Apparently the maximum tolerated dose of streptomycin for guinea pigs inoculated with virulent human tubercle bacilli is about 6000 units per day (approximately 10,000 to 15,000 units per kg) when administered subcutaneously or intramuscularly for 5 to 10 weeks in succession. Undoubtedly the compound has shown some degree of therapeutic effectiveness in the treatment of experimental tuberculosis of guinea pigs in confirmation of previous reports¹⁻³ as based upon the survival rate of treated animals and the results of macroscopic and microscopic examinations of the spleen, liver and lungs for tuberculous lesions in postmortem examinations. In this connection, however, it is to be emphasized that our method of treatment was not maximum since but 2 doses of 1500 units of streptomycin in saline solution were given 4 hours apart with a 3rd dose of 3000 units in peanut oil and 3% beeswax 4 hours later per day, especially since it appears that streptomycin in peanut oil and beeswax is not as readily absorbed from the muscles as penicillin. Under the circumstances diasone by ingestion has yielded somewhat better therapeutic results while combined treatment with streptomycin and diasone has shown better results than observed with either compound alone, suggesting synergistic or additive therapeutic effects.

Summary. Both streptomycin and diasone

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.