

THE IN VITRO AND IN VIVO ACTIVITY OF STREPTOMYCIN AGAINST HEMOPHILUS PERTUSSIS

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Streptomycin (Schatz, Bugie, and Waksman, 1944) has been shown to be efficacious in the treatment of infections due to gram-negative bacteria (Jones *et al.*, 1944; Robinson *et al.*, 1944; Heilman, 1945; Reimann *et al.*, 1945). A determination of the activity of streptomycin against *Hemophilus pertussis* seemed desirable since there is need for an effective agent to be used in the therapy of whooping cough.

IN VITRO ACTIVITY

The *in vitro* susceptibility of various strains of *H. pertussis* to streptomycin was determined by inoculating plates of Bordet-Gengou agar and tubes of a modified liquid Hornibrook medium (Hornibrook, 1939; Verwey and Sage, 1945) containing varying concentrations of the drug. The data are given in table 1. The 4 strains investigated were approximately equally susceptible to the drug, 3 μg^1 per ml being bacteriostatic in Bordet-Gengou agar, slightly greater concentrations being required to prevent growth in the liquid medium.

To determine the bactericidal activity of streptomycin, modified Hornibrook's medium containing 15 μg of streptomycin per ml was prepared. Tubes were inoculated with the 4 strains of *H. pertussis* to give a concentration of 1 billion cells per ml. At intervals samples were removed, diluted beyond the bacteriostatic range of activity of streptomycin, and the viable cells determined by plate counts on Bordet-Gengou agar. No significant decrease in the number of viable cells was found after 6 hours' incubation at 37 C; however, 99.75 per cent of the cells were nonviable after 30 hours. Control cultures without the drug grew normally. It is evident that streptomycin at a concentration of 15 μg per ml was bactericidal.

IN VIVO ACTIVITY

The *in vivo* activity of streptomycin against *H. pertussis* no. 18323² was determined by intracerebral infection of CFI mice, as suggested by Kendrick (1944), and intraperitoneal injections of the drug. Mice weighing from 14 to 16 grams were infected with 40,000 cells of *H. pertussis* contained in 0.04 ml prepared by suspending 24-hour growth on Bordet-Gengou agar plates in modi-

¹ The activity of streptomycin is expressed in terms of the weight of the streptomycin base, one mg of which is equivalent to approximately 1,000 *Escherichia coli* dilution units of Waksman.

² This strain was supplied through the courtesy of Dr. Pearl Kendrick of the Michigan State Department of Health.

fied Hornibrook's medium. Turbimetric methods were used to standardize the suspension, and the virulence of the strain was assayed by serial dilution of the challenge dose. Treatment with streptomycin was initiated immediately after infection, and 0.5 mg was administered intraperitoneally each day (0.125 mg every 6 hours) for a 5-day period. The infected controls received intraperitoneal injections of saline (table 2).

The mean survival time of the animals receiving saline was 3.9 days and was increased to 7.5 days for those receiving 0.5 mg of streptomycin per day. Streptomycin administered at this level markedly increased the survival

TABLE 1
The bacteriostatic effect of streptomycin on Hemophilus pertussis

MG OF STREPTOMYCIN PER ML OF MEDIUM	GROWTH ON BORDET-GENGOU AGAR*				GROWTH IN MODIFIED HORNIBROOK'S MEDIUM†			
	Strain of <i>H. pertussis</i>				Strain of <i>H. pertussis</i>			
	Hass	2,227	2,231	18,323	Hass	2,227	2,231	18,323
6.25	—	—	—	—	0	0	0	0
3.125	—	—	±	±	14	15	13	35
1.56	±	±	±	±	55	26	49	75
0.73	+	+	+	+	122	70	70	170
0.00	+	+	+	+	250	210	220	250

* Recorded as growth or no growth.

† Turbidity as determined on a Klett-Summerson colorimeter.

TABLE 2
Effect of administration of streptomycin at 0.5 mg per day for 5 days
(14- to 16-gram mice)

INFECTING DOSE OF <i>H. PERTUSSIS</i>	TREATMENT	NO. OF MICE	SURVIVAL IN DAYS	PER CENT MORTALITY	MEAN SURVIVAL TIME OF MICE THAT DIED
40,000	0.5 mg per day	10	3, 3, 3, 7, 9, 9, 10, 11, 13, S*	90	7.5
40,000	Saline	10	3, 3, 3, 3, 3, 4, 4, 4, 4, 8	100	3.9
1,250	None	6	5, 6, 8, 9, 9, 10	100	7.8

* S indicates survival for 15 days.

time but did not protect the animals from ultimate death. The lone surviving mouse in the treated group was autopsied on the 15th day and *H. pertussis* was recovered from the brain. Virulence control mice infected with 1,250 bacteria died within 5 to 10 days with a mean survival time of 7.8 days, indicating that the infecting dose given the test animals contained at least 32 minimum lethal doses of *H. pertussis* organisms.

This investigation was extended by a series of experiments in which the infecting dose was decreased and the duration and the intensity of therapy were increased. Mice were infected with 5,000 organisms, and streptomycin was administered over a 10-day period at levels of 1.0 and 2.0 mg per day. Intra-

peritoneal injections of 0.25 and 0.5 mg of streptomycin were made every 6 hours.

As shown in table 3, streptomycin administered at a level of 2.0 mg per day (150 mg per kg per day) protected 50 per cent of the mice against death. However, those that died had a mean survival time of 22 days, the first mortality being on the 19th day. The dosage of 1.0 mg per day permitted 44.5 per cent survival, and the mean survival time of the animals that died was 15.2 days. Mice receiving saline all died between the 5th and 8th day, the mean survival time being 5.5 days. An infecting dose of 312 cells of *H. pertussis* per mouse caused death of 50 per cent of the animals with a mean survival time of 7.4 days. Cultures of *H. pertussis* were recovered from all animals that died during these experiments. All surviving mice were autopsied and cultured on the 38th day after infection; attempts to isolate *H. pertussis* were unsuccessful, and no gross pathological lesions were observed.

TABLE 3

Effect of administration of streptomycin at 2.0 and 1.0 mg per day for 10 days
(13-gram mice)

INFECTING DOSE OF <i>H. PERTUSSIS</i>	TREATMENT	NO. OF MICE	SURVIVAL IN DAYS	PER CENT MORTALITY	MEAN SURVIVAL TIME OF MICE THAT DIED
5,000	2.0 mg per day	10	19, 20, 21, 27, 28, S, S, S, S, S†	50	22.0
5,000	1.0 mg per day	9*	5, 5, 16, 19, 31, S, S, S, S	55.5	15.2
5,000	Saline	10	5, 5, 5, 5, 5, 5, 5, 6, 8	100	5.5
312	None	10	3, 4, 4, 7, 19, S, S, S, S, S	50	7.4

* One mouse died 6 hours after infection because of an inoculation injury.

† S indicates survival for 38 days.

Cultures obtained from the brain of a mouse treated with 2.0 mg of streptomycin per day, and which died 28 days after infection, were tested for resistance to streptomycin and were found to have the same *in vitro* susceptibility as the original culture.

Streptomycin in dosages of 1.0 to 2.0 mg per day protected 50 per cent of the mice and increased considerably the survival times of the animals that died.

These data indicate that further investigations concerning the therapeutic role of streptomycin in *H. pertussis* infection are warranted.

SUMMARY

Streptomycin exhibited *in vitro* bacteriostatic activity against *Hemophilus pertussis* at a concentration of 3 μ g per ml and was bactericidal at 15 μ g per ml.

Infection of mice with 40,000 organisms and treatment with 0.5 mg of streptomycin per day for 5 days resulted in an average survival time approximately twice that of the controls, but an ultimate mortality of 90 per cent of the animals.

Infection with 5,000 organisms and daily treatment with 2.0 mg of streptomycin

cin for 10 days protected 50 per cent of the mice. The mean survival time for those that died was 22 days as compared with 5.5 days for the controls.

Further investigations of the use of streptomycin in the therapy of *H. pertussis* infection are suggested.

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