

Therapeutic Effect of Streptomycin in Experimental Murine Pertussis.*

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Streptomycin, an antibiotic discovered by Waksman and his associates, is produced by the growth of certain strains of *Actinomyces griseus* in a suitable medium.¹ Because it exhibits activity against certain gram-negative organisms and is relatively non-toxic, it seemed to us important to test its value in the treatment of experimental murine pertussis as a preliminary to its use in the human disease. The present report describes the therapeutic effect of streptomycin on the course of the experimental disease in mice.

Materials and Method. A standard type of experimental infection in mice,² used for several years in this laboratory, was employed. It consisted of the intranasal inoculation of groups of standard 3-week-old white Swiss mice with freshly isolated strains of *Hemophilus pertussis*.

The 48-hour growth of *H. pertussis* was scraped from the surface of Bordet medium into 0.85% salt solution, thoroughly mixed, strained through gauze and standardized to contain approximately 10 billion organisms per ml. Four-fold dilutions of this suspension were prepared for the inoculation of subgroups of mice. Under light ether anesthesia, each mouse, held in the upright position, received an intranasal instillation of 0.05 ml of the organism suspension. Usually 5 subgroups, each consisting of 4 mice, were inoculated. The dose of organisms given to the mice of the various subgroups was approximately 125, 31.2, 7.8, 1.9, and 0.47 million respectively.

After inoculation the mice were kept under uniform environmental conditions. Deaths

were recorded daily for a 10-day period, at the end of which period the surviving mice were autopsied and cultures of the lungs made. The method of Reed and Muench³ was used to calculate the 50% endpoint, indicating the minimal lethal dose (MLD) for each group of 20 mice.

Streptomycin hydrochloride (Lot 228) was supplied for these experiments by Merck and Co. through the courtesy of Dr. D. F. Robertson at the request of Dr. S. A. Waksman who had kindly supplied us with material for preliminary experiments. It contained 350 units per milligram of dry material and was dissolved and diluted to appropriate concentration in sterile distilled water. Treatment was instituted 4 hours after the mice were infected.

Sodium penicillin (Lot 299) (Cheplin), in the crystalline form, contained 100,000 Oxford units per 60 mg and was prepared for injection in a similar manner.

Results. Under the conditions of the experiments it is clear that streptomycin was definitely protective against the experimental disease. As indicated by the data (Table I), it required more than 10 times the number of organisms (Schieven Strain) to kill 50% of the treated mice than the untreated. When another strain (Rizzo) was used, the protection ratio was more than 3 to 1 in favor of the treated group. In addition to the difference of the mortality rates between the treated and untreated groups there was a distinct difference between the percentages of surviving mice showing negative lung cultures.

In the first experiment (Table I) 14 (73%) of the 19 surviving treated mice had negative lung cultures, indicating a rapid clearance of the organisms from the lungs as a result of treatment with the antibiotic. Only 2 (18%)

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¹ Schatz, A., Bugie, E., and Waksman, S. A., *Proc. Soc. Exp. Biol. and Med.*, 1944, **55**, 66.

² Bradford, W. L., Brooks, A. M., and Katsamides, C. P., *Yale J. Biol. and Med.*, 1944, **16**, 435.

³ Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

TABLE I.
Therapeutic Effect of Streptomycin in Experimental Murine Pertussis.

	No. of survivors in each sub-group of 4 mice					M.L.D. of millions of organisms	% of survivors with negative lung cultures	Ratio of difference in % to its S.E.
	Dose of organisms in millions							
Exp. I (Schieven Strain)	125	31.2	7.8	1.9	0.47			
Streptomycin*	3	4	4	4	4	>125	73	3.4 to 1
Saline (controls)	0	1	4	3	3	12.7	18	
Exp. II (Rizzo Strain)								
Streptomycin*	4	4	4	4	4	>125	100	2 to 1
Saline (controls)	0	3	3	4	4	39	78	

* Lot 228—0.2 ml (130 units) injected subcutaneously 4 × daily for 4½ days. Total dose for each mouse: 2340 units.

† Lot 228—0.25 ml (250 units) injected subcutaneously 4 × daily for 5 days. Total dose for each mouse: 5000 units.

TABLE II.
Therapeutic Effect of Streptomycin and of Penicillin in Experimental Murine Pertussis.

(Sharpe Strain)	No. of survivors in each sub-group of 4 mice					M.L.D. of millions of organisms	% of survivors with negative lung cultures	Ratio of difference in % to its S.E.
	Dose of organisms in millions							
	125	31.2	7.8	1.9	0.47			
*Streptomycin	4	4	4	4	4	>125	100	4.4-1
†Penicillin	0	0	1	4	2	3.3	29	—
‡Streptomycin and penicillin	4	4	4	4	4	>125	95	4.0-1
Saline (controls)	0	0	1	1	3	1.2	20	

* Lot 228—Each mouse received intraabdominally 0.25 ml (250 units) 4 × daily for 5 days. Total dose: 5,000 units per mouse.

† Lot 299—(Cheplin) Each mouse received intraabdominally 0.25 ml (5 units) 4 × daily for 5 days. Total dose: 100 units per mouse.

‡ Each mouse received intraabdominally 5 units of penicillin and 250 units of streptomycin 4 × daily, for 5 days. Total dose: 100 units of penicillin + 5,000 units of streptomycin.

of 11 untreated survivors had negative cultures. This difference in the percentages of negative cultures obtained from the 2 groups is statistically significant, for the ratio between the difference and its standard error is 3.4 to 1. In the second experiment (Table I) the clearance effect was similar, though not so definite as in the first experiment (ratio: 2 to 1).

The data of Table II concerns a third experiment in which the protective effect of streptomycin was compared with that of penicillin. It is clear that streptomycin was again definitely protective while penicillin

failed. When the 2 antibiotics were combined, the result was similar to that of streptomycin alone.

The protective action of streptomycin as indicated in the above experiments was sufficient to justify a trial in the human disease, studies of which are now in progress.

Summary. Streptomycin exerts a protective therapeutic effect on the course of experimental murine pertussis as indicated by a significant reduction in the mortality rate and the disappearance of the organisms from the lungs of the surviving mice.