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Cytotoxicity of Streptomycin and Streptothricin.

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The present study was done as part of a clinical and laboratory investigation of streptomycin, an antibiotic agent described by Schatz, Bugie, and Waksman.[1] Streptomycin has been found effective in the treatment of a number of experimental infections in laboratory animals.[2-10] In experiments reported by Feldman and Hinshaw[7] and by Feldman, Hinshaw, and Mann,[10] streptomycin was administered to guinea pigs over a long period without causing recognizable toxic reactions or tissue changes. Of several different lots of streptomycin used in the experiments of Feldman and Hinshaw, only one appeared to be toxic, indicating that the reactions observed were probably due to an impurity and not to streptomycin itself.

The tests of cytotoxicity reported in this paper were carried out on a number of samples of streptomycin before they were used for clinical investigation. In addition, a limited number of tests was also done with streptothricin, an agent described by Waksman[11] that is similar in many respects to streptomycin, especially with regard to its selective anti-bacterial activity. Robinson, Graessle, and Smith[5] have reported on the toxicity of streptothricin for mice. In another study they reported that some samples of streptomycin are several times less toxic than streptothricin in acute toxicity tests.[6] Feldman and Hinshaw[12] found streptothricin toxic for guinea pigs when it was given by repeated administration.

Experimental Methods. The tissue culture technic used has been described previously in detail.[13] Young adult male rabbits were used exclusively as a source of tissue, plasma, and serum used in the preparation of cultures. A serum-chick-embryo extract was made by extracting chick embryos of 8 days of incubation with rabbit's serum in the proportion of 1 embryo to 5 cc of serum. Fragments of rabbit's spleen measuring approximately 1 to 2 mm across were selected and were placed in Tyrode's solution in matched groups of 12 fragments each. In each experiment 1 group of 12 fragments was used in the preparation of the control series of cultures and each of the remaining groups comprised a test series. A solution of streptomycin or streptothricin* in Tyrode's solution was added to the tissue extract used in the preparation of the test cultures in an amount that would comprise 1/20

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* Samples of streptomycin and streptothricin were kindly furnished by Dr. Waksman and also by Dr. D. F. Robertson of Merck & Co., Inc. Additional samples of streptomycin were furnished through the kindness of Abbott Laboratories, Parke, Davis & Company, and the Upjohn Company.

TABLE I.
Effect of Different Preparations of Streptomycin on Migration of Macrophages and Growth of Fibroblasts in Cultures of Normal Rabbit's Spleen.

Streptomycin			Macrophages			Fibroblasts		
			Radius of migration, mean ocular micrometer units			Radius of growth, mean planimeter units		
Sample	Potency, units in 1 mg	Dilution × 1000	Control cultures*	Test cultures	% difference	Control cultures	Test cultures	% difference
1	200	1:1	269 ± 63	252 ± 42	— 6	755 ± 30	433 ± 21	*—43*†
		1:2	179 ± 9	192 ± 8	+ 7	1112 ± 63	968 ± 52	—13
		1:4	179 ± 9	174 ± 4	— 3	1112 ± 63	731 ± 66	—34
2	242	1:2	408 ± 21	373 ± 11	— 9	571 ± 31	433 ± 26	*—24*
		1:4	582 ± 20	567 ± 16	— 3	960 ± 27	863 ± 58	—10
3	333	1:2	408 ± 21	353 ± 9	—13	571 ± 31	396 ± 20	*—31*
		1:4	582 ± 20	550 ± 16	— 5	960 ± 27	810 ± 46	*—16*
4	270	1:2	408 ± 21	387 ± 18	— 5	571 ± 31	377 ± 29	*—34*
		1:4	414 ± 17	406 ± 14	— 2	751 ± 54	854 ± 50	+14
5	218	1:2	476 ± 20	382 ± 8	*—20*	927 ± 55	638 ± 30	*—31*
		1:4	414 ± 17	465 ± 20	+12	751 ± 54	784 ± 44	+ 4
6	102	1:2	582 ± 20	521 ± 36	—10	960 ± 27	789 ± 36	*—18*
		1:4	412 ± 17	384 ± 20	— 7	846 ± 30	827 ± 16	— 2
7	220	1:2	534 ± 24	517 ± 16	— 3	843 ± 37	655 ± 61	*—22*
		1:4	412 ± 17	402 ± 14	— 2	846 ± 30	847 ± 28	+ 0.1
8	150	1:2	534 ± 24	521 ± 21	— 2	843 ± 37	588 ± 47	*—30*
		1:4	412 ± 17	392 ± 14	— 5	846 ± 30	804 ± 32	— 5
9	369	1:1	467 ± 22	430 ± 14	— 8	936 ± 37	722 ± 39	*—23*
		1:2	467 ± 22	448 ± 17	— 4	936 ± 37	982 ± 61	+ 5
10	190	1:2	476 ± 20	417 ± 16	—12	927 ± 55	662 ± 15	*—29*
11	185	1:1	468 ± 17	506 ± 15	+ 8	805 ± 53	732 ± 19	— 9
		1:2	468 ± 17	480 ± 11	+ 3	805 ± 53	802 ± 27	+ 0.4
12	250	1:1	275 ± 11	237 ± 8	*—14*	852 ± 63	812 ± 27	— 5
		1:2	275 ± 11	265 ± 18	— 4	852 ± 63	809 ± 38	— 5
13	336	1:1	429 ± 7	364 ± 12	*—15*	730 ± 23	548 ± 28	*—25*
		1:2	429 ± 7	413 ± 10	— 4	730 ± 23	609 ± 30	*—17*
14	100	1:1		‡		730 ± 23	524 ± 23	*—28*
		1:2	429 ± 7	353 ± 12	*—18*	730 ± 23	691 ± 20	— 5

* The value following the ± is the standard error of the mean.
† Values in italics show significant variation from controls.
‡ Precipitate obscured cellular migration.

of the volume of the final tissue culture clot. A similar amount of Tyrode's solution was added to extract used in the preparation of control cultures.

Cultures were placed in D-5 Carrel flasks and consisted of 0.5 cc of heparinized rabbit's plasma, 1.0 cc of serum-chick-embryo extract and 4 explants of spleen which were placed in the medium before clotting occurred. Three flasks were prepared for each experimental condition. Preparations were incubated at 37°C for 5 days. Measurements of the migration of macrophages were made with an ocular micrometer at 96 hours of incubation by a method that has been described.[12] Fibroblastic growth was determined on the fifth day

by projectoscopic methods. The average radius of the growth or migration zone in each group of test cultures was compared with that of the corresponding controls and the results were expressed in terms of percentage of inhibition or stimulation.

Results. Samples of streptomycin obtained from different sources were uniformly low in cytotoxicity (Table I). A concentration of 1:2000 of streptomycin caused a slight but significant inhibition of migration of macrophages in 2 instances and a decrease of fibroblastic growth in 9 of the 14 samples tested. One sample of streptomycin in a concentration of 1:4000 caused a slight but significant inhibition of the growth of fibroblasts but a similar concentration of the other preparations of streptomycin tested did not inhibit growth. Fibroblasts were more sensitive than macrophages to streptomycin except in samples 12 and 14. In the case of sample 14 the drug formed a precipitate in the culture medium. Particulate material frequently has a more toxic effect on macrophages than on fibroblasts because the particles are engulfed by the wandering cells.

The results of tests with 2 samples of streptothricin are recorded in Table II. Both samples were much more toxic for fibroblasts than for macrophages. Leukocytes that were present in the migration zone at 24 hours showed about the same sensitivity to streptothricin as did macrophages. The highest concentrations of the 2 samples of streptothricin that were not toxic for fibroblasts were 1:50,000 and 1:80,000 respectively.

TABLE II.
Effect of Different Concentrations of Streptothricin on Migration of Macrophages and Growth of Fibroblasts in Cultures of Normal Rabbit's Spleen.

Streptothricin		% variation from controls	
Sample	Dilution × 1000	Macrophages	Fibroblasts
1	1:0.5	—100*	—100*
325 units	1:1	— 57	—100*
per mg	1:2	— 45	—100*
	1:10	— 4	—100*
	1:50	+ 3	+ 2
2	1:20	+ 5	—100*
345 units	1:40	+ 6	— 30
per mg	1:60	+ 5	— 28
	1:80	+ 1	— 7

* 100 = complete inhibition of growth.

Comment. The low toxicity of streptomycin in tissue culture is in agreement with its relatively low toxicity for experimental animals.[2-4,7-10] Recently Reimann, Elias, and Price[14] have reported on the use of streptomycin in the treatment of typhoid fever. Serious toxic reactions due to streptomycin did not occur in their series of cases. Because all of the samples tested in the present study were relatively nontoxic, there was very little relation between the cytotoxicity observed and the potency of the preparation of streptomycin in units per milligram. It should be emphasized that the methods employed in the present investigation do not detect histamine activity, pyrogens or numerous other pharmacologic effects that do not interfere directly with the survival and growth of the cells being tested. The cytotoxicity of streptomycin is of about the same order as that of fairly pure penicillin tested by the same method.[15] The relatively low toxicity of streptothricin for leukocytes and macrophages and the fairly high toxicity for fibroblasts demonstrate the advantage of observing the effect of the substance being tested on more than one type of cell. The deleterious effect of streptothricin on fibroblasts is in agreement with the toxicity of streptothricin for guinea pigs observed by Feldman and Hinshaw.

Summary. Several different preparations of streptomycin were tested on cultures of rabbit's spleen and were found to have a uniformly low toxicity for wandering cells and fibroblasts. Streptothricin had a relatively low cytotoxicity for leukocytes and macrophages but showed a fairly high cytotoxicity for fibroblasts.

[14] Reimann, H. A., Elias, W. F., and Price, A. H., *J. A. M. A.*, 1945, **128**, 175.

[15] Heilman, Dorothy H., unpublished data.