one or both gametes can be fatally injured by 20-minute exposure of the adult female to temperatures of 109°-110°F, involving elevation of general body temperature of 5°-6°F. During the second half of the same period like treatment has no observed effect, but, as previously reported[1] another period of susceptibility to heat is experienced on the 3rd day after mating.

14763

Chemotherapeutic Properties of Streptomycin.

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The search for a chemotherapeutic agent effective in the treatment of gram-negative bacterial infections recently led to the isolation of streptothricin.[1] This agent was shown to be active *in vitro*[2,3] and *in vivo*[4,5,6] against a variety of gram-negative bacteria. Streptothricin was also shown to be active *in vitro* against the tubercle bacillus.[7,8]

More recently, a second substance was isolated in crude form from a micröorganism called *Actinomycetes griseus*, which was named streptomycin.[9] Like streptothricin, this substance was found to be active against gram-negative bacteria *in vitro* and *in vivo*.[10] The available data suggest that, with the exception of a few microörganisms, the *in vitro* activity of streptomycin appears to be identical with that of streptothricin. Our findings show that a noteworthy difference between these two substances seems to be in the greater activity shown by streptomycin in infections produced by certain pathogenic bacteria, and also in the lower toxicity of streptomycin.

The present report is mainly concerned with the comparative value of streptomycin and streptothricin as chemo-therapeutic agents in the treatment of gram-negative and gram-positive infections in mice.

Materials and Methods. With the exception of the streptomycin, the materials and methods of the *in vitro* and *in vivo* assays have been reported in previous communications, and will therefore not be described in detail here.[6] The streptothricin W and streptothricin F used were the same as those reported in a recent paper.[11] The streptomycin was kindly supplied by Dr. S. A. Waksman of Rutgers University, and Drs. M. Tishler and R. Denkewalter of the Research Laboratories of Merck & Co., Inc. The potency of this substance was 27 to 100 units per mg of solid, one unit being that quantity of streptomycin which will just inhibit a given strain of *E. coli* in 1 ml of nutrient broth or agar, as described by Waksman *et al.*[10] The streptothricin unit differs somewhat from the streptomycin unit and has been described by Foster *et al.*[3] The inhibitory action of these substances on the growth of bacteria and fungi *in vitro* was determined by incorporating the drugs in agar and streaking the surface of the

1 Waksman, S. A., and Woodruff, H. B., *J. Bact.*, 1940, **40**, 581.

2 Waksman, S. A., and Woodruff, H. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 609.

3 Foster, J. W., and Woodruff, H. B., *Arch. Biochem.*, 1943, **3**, 241.

4 Robinson, H. J., Thesis, Rutgers University, 1943.

5 Robinson, H. J., Graessle, O. E., and Smith, D. G., *Science*, 1944, **99**, 540.

6 Robinson, H. J., and Smith, D. G., *J. Pharm. and Exp. Therap.*, in press.

7 Foster, J. W., and Woodruff, H. B., in press.

8 Schatz, A., and Waksman, S. A., in press.

9 Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.

10 Jones, D., Metzger, H. J., Schatz, A., and Waksman, S. A., *Science*, 1944, **100**, 103.

11 Robinson, H. J., Graessle, O. E., and Silber, R. H., *J. Pharm. and Exp. Therap.*, in press.

TABLE I.
Acute Subcutaneous Toxicity for Mice, Streptomycin and Streptothricin.

Drug	No. of mice	Dose in units per 20 g	No. dead Time in days 1	2	3	4	5	6	7	8	9	10	% dead
Streptothricin	15	1250	0	0	0	0	0	0	0	0	0	0	0
"	15	2500	0	0	0	0	2	0	2	0	2	0	40
"	15	5000	0	0	2	2	4	3	2	2	–	–	100
Streptomycin (low potency)	20	1250	0	0	0	0	0	0	0	0	0	0	0
" " "	25	2500	6	1	0	0	0	0	0	0	0	0	28
" " "	20	5000	16	2	0	0	0	0	0	0	0	0	90
Streptomycin (high potency)	15	5000	0	0	0	0	0	0	0	0	0	0	0
" " "	15	7500	0	0	0	0	0	0	0	0	0	0	0
" " "	15	10,000	0	0	0	0	0	0	0	0	0	0	0
" " "	10	15,000	9	0	0	0	0	0	0	0	0	0	90

agar with the test organism. The rate at which streptomycin killed bacteria was determined in defibrinated rabbit blood or broth, using the rotating rack technic as described in earlier papers.[12]

Acute Toxicity. A summary of the acute toxicity tests performed with streptomycin and streptothricin is presented in Table I. The results show that certain batches of streptomycin are considerably less toxic than streptothricin. A further difference between the two substances appeared to be that death following the injection of streptomycin occurs within 48 hours after the injection, whereas in the case of streptothricin W the majority of deaths occur after the third day. The toxic signs manifested by streptothricin have been reported elsewhere, and therefore will not be described here.[11] The visible responses of mice to toxic doses of streptomycin consisted of increased activity, marked dypsnea, and in some animals death due to respiratory failure. It was observed that the more toxic preparations of streptomycin lowered the blood pressure of rabbits in the same manner as streptothricin.[11] On the other hand, the least toxic lot of streptomycin had no effect upon the blood pressure of rabbits, even in doses as large as 12,000 units per kg. These findings suggest that under certain conditions streptomycin may be contaminated with a toxic factor which is lethal for mice, and causes a precipitous fall in blood pressure of rabbits.

Results in vitro—*Agar Plate.* The results of the bacteriostatic tests are summarized in Table II and show that the spectrum of activity of streptomycin is in many respects identical with that of streptothricin. Both substances show remarkable activity against both gram-positive and gram-negative pathogens.

TABLE II.
Bacteriostatic Action of Streptomycin and Streptothricin.
Agar Plate Method.

Organism	Factors for inhibition Streptomycin	Streptothricin
**E. coli* "W"	1	1
E. coli "C"	1	0.5
Strep. hemolyticus MIT	> 16	> 16
Strep. hemolyticus M	2	16
Strep. viridans	> 16	> 16
Strep. lactis	4	4
Staph. aureus SM	0.5	4
Staph. aureus FDA	1	1
Staph. aureus SD	> 16	1
Staph. aureus 155	2	4
Diplo. pneumoniæ II M	8	4
B. mycoides	0.5	> 16
B. subtilis	0.25	0.5
E. typhi	1	0.25
S. aertrycke	4	2
S. enteritidis	0.5	0.5
S. schottmülleri	2	2
B. flexneri II	0.25	1
B. sonne	1	1
P. leptisepticum	0.5	0.5
B. proteus S	4	16
B. proteus	4	8
B. pyocyaneus II	> 16	> 16
B. pyocyaneus III	> 16	> 16
A. aerogenes	0.5	1
Cl. welchii WX	>104	>104
Cl. tetani	>104	>104
Cl. septicum	>105	52
Cl. sordelli	>105	>104

* 7.5 units per 1 cc of agar required to inhibit *E. coli* "W."

[12] Robinson, H. J., *J. Pharm. and Exp. Therap.*, 1943, **77**, 70.

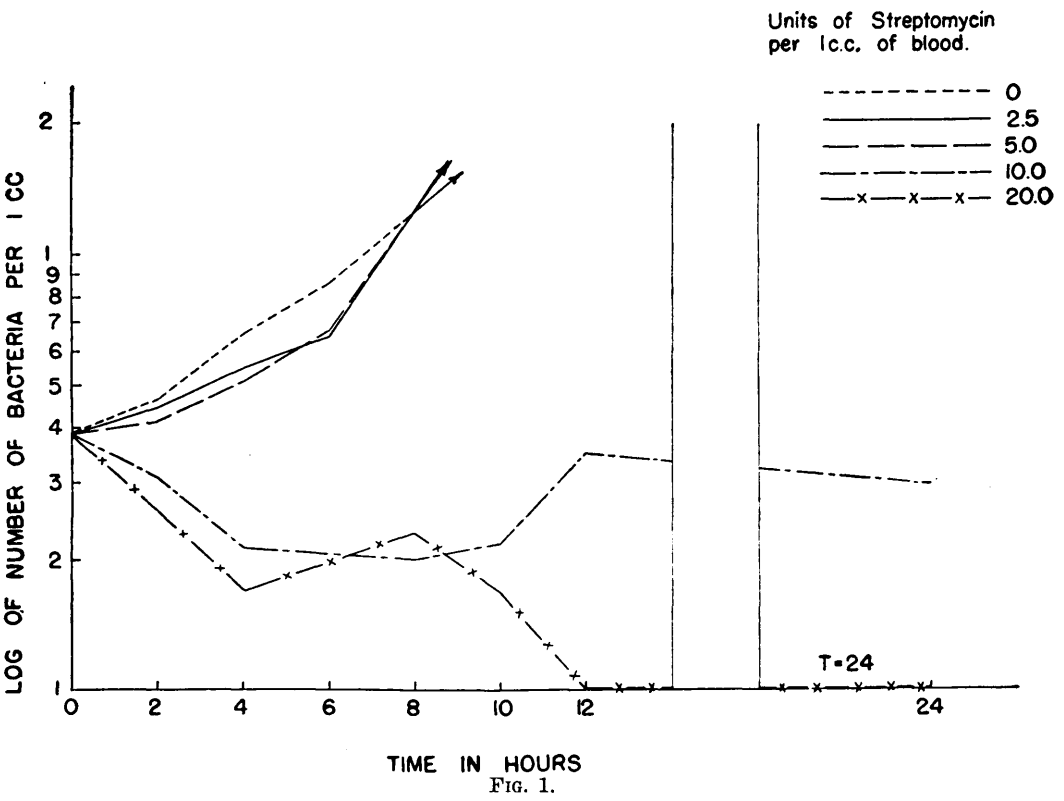

FIG. 1.

However, a notable difference between streptomycin and streptothricin was noted in their respective action against certain bacterial strains, even within the same species. Thus, streptomycin was 8 times more effective than streptothricin against *Staph. aureus SM*, but 16 times less effective against *Staph. aureus SD, B. mycoides, B. flexneri, B. proteus, A. aerogenes,* and certain strains of staphylococci and streptococci appeared to be more sensitive to streptomycin than to streptothricin. Neither of the substances was very active against the gram-positive anaerobes.

Pathogenic and saprophytic fungi were much more susceptible to the action of streptothricin (Table III). In concentrations of 250 units per cc of agar the latter was fungistatic for most strains, whereas the same concentration of streptomycin appeared to be without effect. When higher concentrations of streptomycin were employed, however, streptomycin also showed fungicidal properties.

TABLE III.

Bacteriostatic Action of Streptomycin and Streptothricin Against Fungi.
Agar Plate Method—Sabouraud's Agar.

Organism	No. of units per cc agar required to cause inhibition	
	Streptomycin	Streptothricin
Aspergillus niger MF II	>4,000	250
Penicillium chrysogenum MF 56	>4,000	250
Cryptococcus neoformans No. 3709	>4,000	250
Epidermophyton inguinale	>4,000	1,000
Microsporum canis No. 232	4,000	1,000
Sporotrichum schenki No. 7017	>4,000	250
Trichophyton gypseum	3,500	500
Trichophyton interdigitale No. 640	4,000	1,000

Cultures were incubated at 29°C for 14 days.

Rotating Rack Technic. When the test organism was cultured in blood or broth in the presence of streptomycin or streptothricin,

TABLE IV.

Efficacy of Streptomycin in Mice Infected with *S. schottmülleri*. Comparison of Intravenous, Intraperitoneal, Subcutaneous, and Oral Therapy.

Organism: *Salmonella schottmülleri.*
Age of Culture: 6 hours.
Infection: 0.5 cc of a 10^{-5} dilution in 4% mucin.
Therapy: Streptomycin given intravenously, intraperitoneally, subcutaneously, or orally immediately after inoculation.

No. of mice	Drug	Units per dose	No. doses per day	Culture dilution	No. surviving in days 1	2	3	4	5	6	7	8	9	10	% survival
				Therapy: A single intravenous dose.											
10	Streptomycin	3.125	1	10^{-5}	1	0	0	0	0	0	0	0	0	0	0
10		6.25	1	"	2	1	1	0	0	0	0	0	0	0	0
10		12.5	1	"	3	3	1	1	1	1	1	1	1	1	10
10		25.0	1	"	5	2	2	2	2	1	1	1	1	1	10
10		50.0	1	"	9	7	6	6	6	6	6	6	6	6	60
10		100.0	1	"	10	10	10	10	10	9	9	9	9	9	90
10		200.0	1	"	10	10	10	10	10	10	10	10	10	10	100
				Therapy: A single intraperitoneal dose.											
30	Streptomycin	3.125	1	10^{-5}	5	4	3	3	2	2	2	2	2	2	6.7
30		6.25	1	"	13	6	4	2	2	2	2	2	2	2	6.7
30		12.5	1	"	25	21	20	19	18	18	17	17	16	16	52.8
30		25.0	1	"	28	26	26	26	26	26	24	24	23	23	75.9
30		50.0	1	"	29	29	29	29	29	29	29	29	29	29	96.7
				Therapy: A single subcutaneous dose.											
30	Streptomycin	6.25	1	10^{-5}	3	3	3	3	3	3	3	3	3	3	9.9
30		12.5	1	"	7	6	5	4	3	3	2	2	2	2	6.6
30		25.0	1	"	16	13	10	9	6	6	6	6	6	6	19.8
30		50.0	1	"	28	28	28	28	27	27	26	26	26	26	85.8
30		100.0	1	"	30	30	29	29	29	29	29	29	29	29	96.7
				Therapy: A single oral dose.											
10	Streptomycin	93.75	1	10^{-5}	4	2	2	2	0	0	0	0	0	0	0
10		187.5	1	"	1	1	1	0	0	0	0	0	0	0	0
10		375.0	1	"	2	0	0	0	0	0	0	0	0	0	0
10		750.0	1	"	3	2	2	2	1	1	1	1	1	1	10
10		1500.0	1	"	9	7	7	7	6	6	6	6	6	6	60
10		3000.0	1	"	10	10	9	9	8	8	8	8	8	8	80
				Therapy: None.											
40	Controls	—	—	10^{-5}	0	0	0	0	0	0	0	0	0	0	0
20		—	—	10^{-6}	6	2	2	1	1	1	1	1	0	0	0
20		—	—	10^{-7}	12	7	7	7	6	5	4	3	3	3	15
20		—	—	10^{-8}	15	8	7	5	5	5	5	4	4	4	20

and the rate of killing estimated by counting the surviving organisms at frequent intervals, it was found that concentrations of 20 units of either drug per cubic centimeter of blood produced a gradual killing effect upon *S. schottmülleri* which sterilized the culture within 12 hours (Fig. 1). Concentrations of 10 units per cc exerted a bacteriostatic effect, whereas lower concentrations had little inhibitory effect. Similar findings were obtained with other organisms, including *E. coli, S. aertrycke,* and *E. typhi.*

Microscopic examination of the cultures containing streptomycin or streptothricin revealed an elongation of the majority of the cells such as that reported following exposure to penicillin,[13], streptothricin,[3] and the sulfonamides.[14] The morphological changes were induced at dilutions far greater than the minimal inhibitory dilution, and suggest that very small amounts of streptomycin and streptothricin may influence the normal rate of division and hence the pathogenicity of the organism.

Results in vivo. Since the *in vivo* findings with a number of bacterial strains of the colon-typhoid and salmonella group were essentially the same, only the results with one bacterial strain are presented in Table IV. Single doses of 50 to 100 units of streptomycin

[13] Smith, L. D., and Hay, T., *J. Franklin Inst.*, 1942, **233**, 598.

[14] Lockwood, J. S., *J. Immun.*, 1938, **35**, 155.

TABLE V.
Efficacy of Streptomycin in Mice Infected with *S. schottmülleri*. Subcutaneous Therapy.

Organism: *Salmonella schottmülleri.*
Age of Culture: 6 hours.
Infection: 0.5 cc of a 10^{-5} dilution in 4% mucin.
Therapy: Streptomycin or streptothricin given subcutaneously immediately after bacterial inoculation.

No. of mice	Drug	Units per dose	No. doses per day	Culture dilution	No. surviving in days 1	2	3	4	5	6	7	8	Survival %
				Therapy: A single dose.									
10	Streptomycin	12.5	1	10^{-5}	2	2	2	2	2	1	1	1	10
10		25.0	1	"	8	8	8	7	7	7	7	7	70
10		50.0	1	"	10	10	10	9	9	9	9	9	90
10		100.0	1	"	9	9	9	9	9	9	9	9	90
10	Streptothricin	12.5	1	10^{-5}	4	3	2	2	2	2	2	2	20
10		25.0	1	"	5	4	4	4	4	4	4	4	40
10		50.0	1	"	10	10	10	9	9	9	9	9	90
10		100.0	1	"	10	10	10	10	10	10	10	10	100
				Therapy: Every 6 hr over a 24-hr period.									
20	Streptomycin	12.5	4	10^{-5}	15	12	12	12	12	11	11		55
20		25.0	4	"	20	19	18	18	18	18	18	18	90
20		50.0	4	"	20	20	20	20	20	20	20	20	100
10	Streptothricin	12.5	4	10^{-5}	9	5	4	4	4	3	3	3	30
10		25.0	4	"	10	10	10	10	9	9	9	9	90
10		50.0	4	"	10	10	10	10	10	10	9	9	90
10		100.0	4	"	10	10	10	10	10	10	10	10	100
				Therapy: None.									
20	Controls	—	—	10^{-5}	0	0	0	0	0	0	0	0	0
10		—	—	10^{-6}	6	4	3	3	3	3	3	3	20
15		—	—	10^{-7}	10	8	7	6	6	6	6	5	33
15		—	—	10^{-8}	12	10	10	10	10	9	9	9	66

per mouse given intravenously or subcutaneously immediately after the bacterial inoculation, were sufficient to protect a large percentage of the mice from a lethal infection of *S. schottmülleri.* When given by intraperitoneal injection, the drug was even more efficacious than following subcutaneous or intravenous administration. As reported for streptothricin,[5] streptomycin was found to be much less effective by mouth than following parenteral administration.

Multiple doses of streptomycin given every 6 hours over a 24-hour period appeared to afford essentially the same protection as equal quantities of the drug given as a single dose immediately after the bacterial inoculation. Essentially the same results were obtained with streptothricin (Table V).

Infections produced by certain gram-positive bacteria were found to be somewhat more resistant to streptomycin than were the infections caused by the foregoing gram-negative bacteria. Nevertheless, infections caused by *Diplococcus pneumoniæ* and *Staphylococcus aureus* were readily controlled by adequate doses of streptomycin (Table VI.) Under the same conditions, streptothricin was not efficacious.

Discussion. Streptomycin, like streptothricin, would seem to offer promise as a chemotherapeutic agent for the treatment of a variety of bacterial infections. Streptomycin seems to possess some advantages over streptothricin, particularly from the point of view of toxicity and its action *in vivo* against gram-positive bacteria. Although some batches of streptomycin were as toxic as streptothricin, the findings suggest that under the proper conditions a relatively non-toxic preparation can be obtained. The toxic signs manifested by mice injected with lethal doses of streptomycin were in many respects similar to those produced by streptothricin F, a preparation recently described by Robinson, Graessle and Silber[11] which was obtained from a medium containing corn steep liquor. The nature of the toxic signs produced by streptomycin and streptothricin F appears to be identical and

TABLE VI.
Efficacy of Streptomycin in Mice Infected with *D. pneumoniæ*. (Subcutaneous Therapy.)

Organism: *Diplococcus pneumoniæ* Type I, No. 37.
Age of Culture: 6 hours.
Infection: 0.5 cc of a 10^{-6} culture dilution in broth.
Therapy: Streptomycin or streptothricin given subcutaneously immediately after bacterial inoculation.

No. of mice	Drug	Units per dose	No. doses per day	Culture dilution	No. surviving in days 1	2	3	4	5	6	7	8	Survival %
				Therapy: A single dose.									
10	Streptomycin	50	1	10^{-6}	1	0	0	0	0	0	0	0	0
10		100	1	"	1	0	0	0	0	0	0	0	0
20		200	1	"	1	0	0	0	0	0	0	0	0
20		400	1	"	9	2	2	1	1	1	1	1	5
20		800	1	"	19	6	6	5	5	5	5	5	25
20		1600	1	"	20	20	20	20	20	20	20	20	100
10		3200	1	"	10	10	10	10	10	10	10	10	100
10		6400	1	"	10	10	10	10	10	10	10	10	100
				Therapy: A single dose.									
20	Streptothricin	200	1	10^{-6}	0	0	0	0	0	0	0	0	0
20		400	1	"	3	0	0	0	0	0	0	0	0
20		800	1	"	14	2	2	0	0	0	0	0	0
20		1600	1	"	19	11	8	5	3	3	2	2	10
				Therapy: None.									
30	Controls	—	—	10^{-6}	0	0	0	0	0	0	0	0	0
20		—	—	10^{-7}	2	0	0	0	0	0	0	0	0
20		—	—	10^{-8}	4	0	0	0	0	0	0	0	0
20		—	—	10^{-9}	6	0	0	0	0	0	0	0	0

seems to be due to a histamine-like substance present in the toxic preparations. To date, sufficient quantities of streptomycin have not been available for extensive toxicological and pharmacological studies. Until such studies are completed, the value of this substance for the treatment of bacterial disease in animals and man cannot be fully evaluated. Although streptomycin appears to have a number of advantages over streptothricin, the latter is much more effective against fungi.

Summary. Streptomycin seems to possess some advantages over streptothricin particularly from the point of view of toxicity and its greater action against certain gram-negative and gram-positive bacteria *in vivo*. Although certain batches of streptomycin appear to have the same order of toxicity as streptothricin, the findings suggest that under proper conditions a relatively non-toxic preparation can be obtained. Streptothricin seems to be much more effective *in vitro* against pathogenic fungi than streptomycin.

14764

Effect of Feeding Dried Milk on Production of Liver Cancer by *p*-Dimethylaminoazobenzene.

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Dietary factors greatly influence the formation of liver cancer by *o*-aminoazobenzene or *p*-dimethylaminoazobenzene (butter yellow). Experiments have shown that the incidence of liver cancer in rats caused by these chemicals is definitely reduced when the basal diet consists of wheat,[1] rye,[2] or millet seed[3] instead of rice.

[1] Ando, T., *Gann*, 1938, **32**, 252.

[2] Maisin, J., *Bull. Assoc. franc. p. l'étude du cancer*, 1940, **29**, 6.