TWO ANTIBIOTICS PRODUCED BY A STREPTOMYCES

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Received for publication March 12, 1947

The genus *Streptomyces* has gained considerable recognition in the field of antibiotics. Outstanding members of the genus to date are *Streptomyces antibioticus* (Waksman and Woodruff, 1941), *Streptomyces lavendulae* (Waksman and Woodruff, 1942), and *Streptomyces griseus* (Shatz *et al.*, 1944), which elaborate actinomycin, streptothricin, and streptomycin, respectively. The streptomyces isolate studied in this work bears a resemblance to *S. lavendulae* but has morphological and biochemical characteristics that differentiate it from this described species. Moreover, by biological and chemical tests, the antibiotic activity of the isolate has been found to result from a mixture of at least two antibiotic substances. Subsequent chemical fractionation has yielded one material in relatively pure form. However, the second active fraction has not been fully separated from the first. A more complete study of the purified fractions will be reported later.

This paper covers the production of antibiotic activity, the preparation of crude concentrates, activity tests, and preliminary attempts to separate the active components. Some of the methods and techniques applied to determine the dual nature of the antibiotic activity may be of service to others confronted with an apparently new biologically active substance.

EXPERIMENTAL RESULTS

Isolation, description, and identification of the streptomyces isolate. The streptomyces was isolated by one of us (C. F.) from a contaminant on a petri plate seeded with *Brucella abortus*. The presence of the contaminant was characterized by a large zone of inhibition surrounding the streptomyces colony. This contaminant was picked and subjected to extensive study for the production of the antibiotic material. Extreme care was exercised at the outset to ensure a pure culture, but to ensure further its purity, single colonies were picked from agar plate dilutions of spores of the mother culture and propagated individually to afford sufficient inoculum for shake cultures. Following growth of the cultures in shake culture flasks for 5 days, the broths from 20 such spore isolates were assayed for potency by the *Bacillus subtilis* cup assay method and also subjected to bacterial spectrum analysis by the agar streak method with streptomycin-resistant test organisms (methods described below). Although the yield of active material varied appreciably among the 20 cultures, their bacterial spectra were similar. It was concluded that the streptomyces culture was pure insofar as the antibiotic activity of individual spore cultures was concerned.

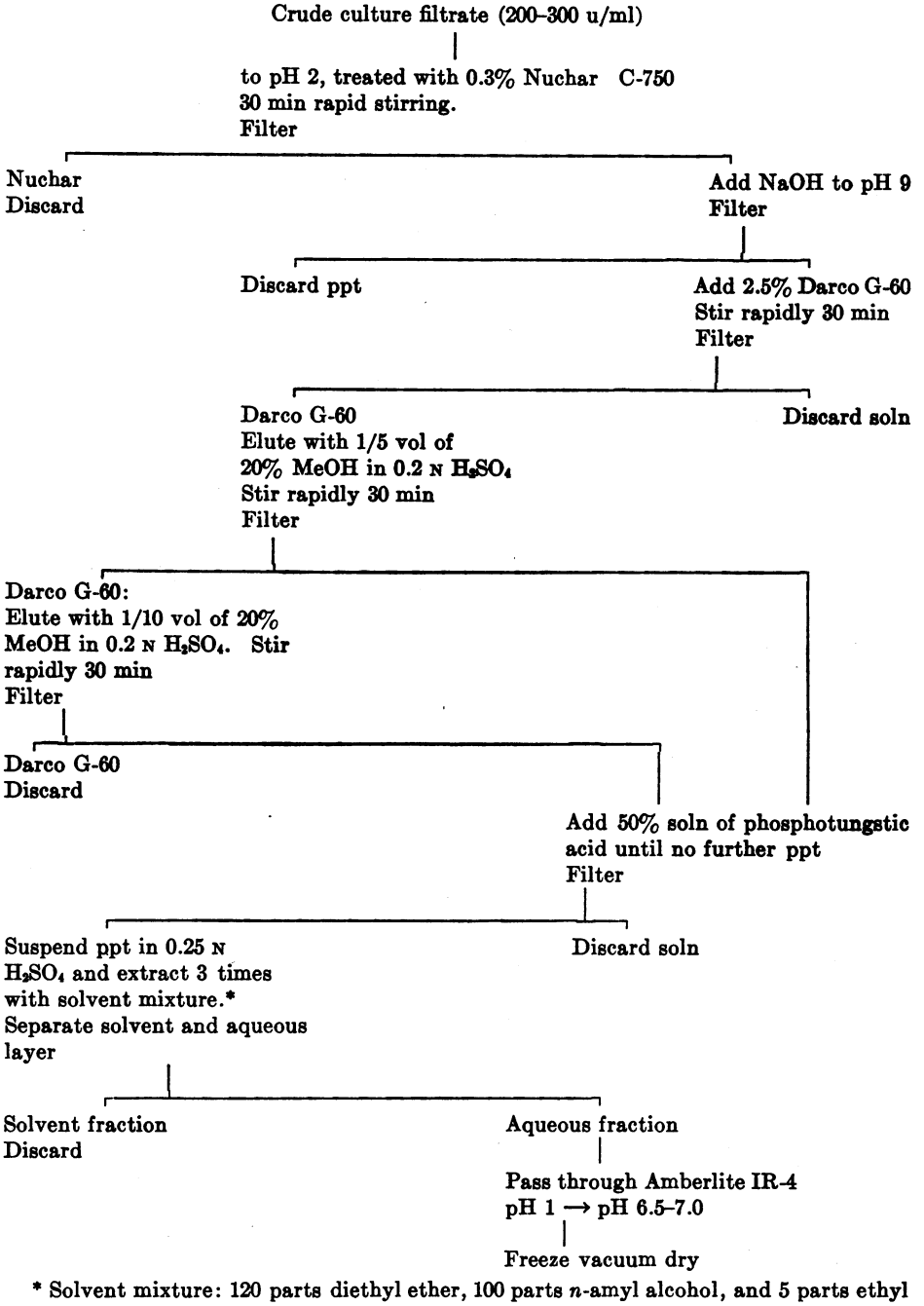
Together with *S. lavendulae* (Waksman no. 10 strain) and *S. griseus* (Waksman

no. 10 strain), the newly isolated streptomyces was examined systematically for its morphological and biochemical characteristics according to the procedures of Waksman (1919). To ensure stabilized cultures, the three organisms were first maintained on sterile soil for 5 weeks. The *S. lavendulae* and *S. griseus* cultures were found to coincide closely with the descriptions presented by Waksman. However, the newly isolated culture did not sufficiently resemble any species in Waksman's key to justify ascribing a known species name to it.

On the diagnostic media the isolate displayed distinct differences from *S. lavendulae* in type and amount of growth as well as in soluble pigment production. On Dorset's egg medium *S. griseus* produced a wrinkled, yellow growth, formed no soluble pigment, and caused no liquefaction; *S. lavendulae* produced a wrinkled purple growth, formed a pink soluble pigment, and caused liquefaction; whereas the streptomyces isolate produced a clay-colored growth, formed a brownish-drab soluble pigment, and caused no liquefaction. On Loeffler's blood serum *S. griseus* appeared as a yellow growth, produced no soluble pigment, and caused liquefaction; *S. lavendulae* appeared as a light cinnamon-colored growth, produced no soluble pigment, and caused no liquefaction; the isolate appeared as a clay-colored growth, produced a pink soluble pigment, and showed questionable liquefaction. Soluble pigments were produced on glycerol nitrate by *S. griseus* (yellow) and by *S. lavendulae* (brown), but not by the isolate. On this medium the aerial hyphae of *S. griseus* were water green, of *S. lavendulae* lavender, and of the isolate pallid mouse gray. Moreover, sharp differences were observed in the structure of aerial hyphae. *S. griseus* produced straight sporogenous hyphae, *S. lavendulae* slightly coiled hyphae, and the isolate tightly coiled hyphae. The most striking biochemical difference was the failure of the isolate to liquefy gelatin or peptonize milk after 1 month of incubation at 25 C.

Assay and production of the antibiotic material. Solutions of the antibiotic were assayed by the "penicylinder" plate method, with *B. subtilis* Marburg as the test organism. The diameters of the zones of inhibition, when plotted against the logarithm of the dosage, exhibited a linear relation, and the slope of the curves was similar to that obtained with streptomycin. This relation was found also when the test organism was *B. subtilis* ATCC 6633, *B. mycoides* (Waksman), and *Serratia marcescens* (Waksman). Accordingly, in evaluating the activity of solutions of the new antibiotic, all samples were assayed against a streptomycin standard, and the values were expressed as equivalent to streptomycin units assayed against *B. subtilis*. For routine assays, large plates of the type described by Beadle *et al.* (1945) were employed. The streptothricin unit also was standardized to the foregoing streptomycin unit.

Propagation of the streptomyces for production of the antibiotic was almost exclusively by submerged culture in shake culture flasks (300 to 400 ml of liquid) or in aerated fermenters (10 liters and 200 liters of liquid). When a medium containing soy flour hydrolyzate, glucose, and salts or a medium containing soy flour, corn steep, glucose, and salts was used, yields of 200 to 300 units per ml were secured consistently in 4 to 5 days. Variation of incubation temperatures between 24 and 29 C did not noticeably change the rate of formation or the



* Solvent mixture: 120 parts diethyl ether, 100 parts *n*-amyl alcohol, and 5 parts ethyl alcohol.

maximum yield of the antibiotic material. Incubation at 37 C slightly depressed the final yield.

Purification of antibiotic material. Spot tests on clarified culture filtrate showed the active materials to be heat-labile (boiling or autoclaving at 15 pounds' steam pressure for 30 minutes), acid-stable (pH 1 for 2 hours), alkali-labile (pH 11.5 for 2 hours), adsorbable on activated carbon, precipitable by phosphotungstic but not by trichloracetic acid, and not extractable at pH 3.0 or 8.1 by ether, chloroform, amyl acetate, or ethyl acetate.

On the basis of the foregoing and subsequent data, the accompanying method of processing was evolved.

The foregoing purification procedure, when culture filtrate of 200 to 300 u per ml is employed, has given a recovery of 40 to 55 per cent of the activity, as 400 to 600 u per mg material. A 190-liter batch gave a recovery of 39 per cent at 592 u per mg; the only other large run to date, 230 liters, gave a recovery of 53 per cent at 483 u per mg.

For convenience the antibiotic (when considered as a mixture of active fractions) will be designated in the remainder of the paper as "F."

Comparison of antibiotic "F" with streptothricin and streptomycin. Bacterial spectrum analysis of the three antibiotics by the agar streak method (4 per cent tryptose agar, Difco) showed that the inhibitory action of antibiotic "F" resembled that of streptomycin, but "F" resembled streptothricin in its effect on the Bodenheimer organism (table 1).

A comparison was therefore made with this organism on a less nutritious medium, nutrient agar (as recommended for the streptomycin test by the Food and Drug Administration, July, 1946). The Bodenheimer organism was found to withstand more than 1,000 u per ml of streptomycin, whereas it was completely inhibited by 1 u per ml of antibiotic "F." A number of preparations of antibiotic "F" were tested in this way, and the effect was found to be consistent.

The three antibiotics were compared by the cysteine inactivation, iodine regeneration procedure of Denkelwater *et al.* (1945). According to these workers, streptomycin may be completely inactivated with cysteine and subsequently reactivated by treatment with iodine, but the activity of streptothricin, on the other hand, is not affected by cysteine. Table 2 shows the results of representative tests with cysteine and iodine. Some samples of antibiotic "F" responded to iodine regeneration, whereas others remained essentially unchanged. Tests on a large number of samples of the three antibiotics showed, on the average, that streptomycin samples were inactivated by cysteine with recovery to 90 per cent of the original activity on iodine treatment; streptothricin samples dropped to 75 per cent with cysteine and recovered to 95 per cent with iodine; and samples of antibiotic "F" dropped to 50 per cent with cysteine and recovered to 80 per cent with iodine. However, there was wide discrepancy among samples of antibiotic "F," particularly in regard to the percentage regeneration with iodine.

To determine whether bacteria made resistant to streptomycin would also be resistant to the new antibiotic, and vice versa, *B. subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were carried through eight transfers on nutrient agar (FDA streak plate agar) containing increasing con-

centrations of one antibiotic. The resulting resistant cultures were tested against the other antibiotic. All the organisms developed resistance more readily against streptomycin than against "F." The data are summarized in table 3. *B. subtilis*, *E. coli*, and *P. aeruginosa* were modified to withstand 1,000 u per ml of streptomycin, the highest concentration of antibiotic used. Only *P. aeruginosa*

TABLE 1

Bacterial spectra of streptomycin, streptothricin, and antibiotic "F" (tryptose agar medium)

Numbers represent amount of growth compared with control cultures: 4 = equivalent to control; 0 = no growth. Incubation at 37 C for 48 hours

ORGANISM	STREPTOMYCIN u/ML					STREPTOTHRICIN u/ML					ANTIBIOTIC "F" u/ML				
	0.3	1	3	10	30	0.3	1	3	10	30	0.3	1	3	10	30
B. subtilis Waksman	3	2	1	0	0	4	3	3	0	0	3	1	1	0	0
E. coli Merck	3	2	2	1	0	4	4	3	2	1	3	2	2	1	0
M. phlei ATCC 355	4	1	0	0	0	4	4	4	1	0	4	1	0	0	0
B. megatherium	4	4	2	0	0	4	4	4	4	2	3	3	1	0	0
S. aureus 209	4	4	2	1	0	4	4	4	3	2	4	3	2	1	0
Brucella abortus 7705	4	1	0	0	0	4	4	3	0	0	4	1	0	0	0
Brucella abortus 19	4	1	0	0	0	4	4	3	1	0	4	1	0	0	0
Brucella melitensis	4	1	0	0	0	4	4	4	1	0	4	2	0	0	0
Brucella suis	4	1	0	0	0	4	4	4	1	0	3	1	0	0	0
B. subtilis 6633	3	0	0	0	0	4	3	1	0	0	2	0	0	0	0
E. coli Waksman	3	2	2	1	0	4	4	3	2	1	3	2	1	1	0
B. mesentericus	3	3	2	1	0	4	4	4	4	4	3	3	1	1	0
S. aureus 24T	4	3	3	2	1	4	4	4	3	2	4	3	2	2	1
C. diphtheriae	3	1	0	0	0	4	4	4	4	1	4	1	0	0	0
K. pneumoniae	4	4	1	0	0	4	4	4	4	4	3	3	1	0	0
P. aeruginosa	4	4	4	3	2	4	4	4	3	1	4	4	4	4	2
S. enteriditis	3	2	2	1	0	4	3	3	2	1	2	2	1	1	0
B. mycoides Waksman	3	3	2	1	0	4	4	4	4	4	4	3	2	1	0
S. hemolyticus	4	4	2	0	0	4	4	4	4	1	4	3	2	0	0
B. subtilis Marburg	4	4	4	3	2	4	4	4	3	3	3	3	2	2	1
S. albus	4	4	1	0	0	4	4	4	4	0	4	4	1	0	0
S. marcescens Waksman	4	3	2	1	0	4	4	4	3	2	3	3	2	1	1
E. typhi	4	4	3	2	1	4	4	3	2	1	4	3	1	0	0
S. schottmuelleri	4	4	3	2	1	4	4	3	2	1	4	3	2	1	0
S. suipestifer	4	4	3	2	1	4	4	3	3	1	4	2	2	0	0
*Bodenheimer**	4	4	4	4	4	4	4	4	1	1	4	4	3	1	0

* This organism was isolated by Dr. Bodenheimer of the College of Physicians and Surgeons, Columbia University, New York City.

became resistant to 1,000 u per ml of "F"; the remaining three species withstood not more than 1 u per ml after eight transfers.

When the foregoing experiment was repeated with a different preparation of "F" (organisms carried through 14 transfers in the presence of the antibiotics), it was observed that not only were "F"-resistant organisms generally as resistant to streptomycin, but streptomycin-resistant organisms were (with the exception of *B. subtilis*) equally resistant to antibiotic "F" (table 3).

TABLE 2

Cysteine inactivation, iodine regeneration of antibiotics

SAMPLE	UNTREATED u/ML*	AFTER CYSTINE		AFTER I_2 REGENERATION	
		u/ml	% of original	u/ml	% of original
F-3	484	315	65	389	80
Streptomycin	550	42	8	513	93
F1-2A-10	550	306	56	516	94
F3-1	506	354	70	504	100
Streptomycin	597	30	5	582	98
Streptothricin	220	178	81	252	114
F31-35-7	400	111	28	295	74
F1-2A-10A	705	362	51	414	59
Streptomycin	160	tr		134	84
F31-35-7	400	82	20	308	77
F4-7-3C-10-2	215	96	45	212	98
F1-2A-10A	705	447	64	447	64
Streptomycin	160	tr		144	90
Streptomycin	427	tr		367	86
Streptothricin	220	165	75	216	98

* For ease of comparison, all original solutions are based on 1 mg per ml, so that figures in this column also indicate u per mg (i.e., degree of purity).

TABLE 3

Comparison of cultures made resistant to streptomycin (S) and antibiotic "F" by serial transfer

ORGANISM	TREATMENT	MAXIMUM CONC. PERMITTING GROWTH, u/ML			
		8 transfers (EXPT. 1)		14 transfers (EXPT. 2)	
		S	"F"	S	"F"
B. subtilis 6633	Unmodified	0.1	0.1	1.0	1.0
	S-resistant	1,000	0.1	1,000	30
	F-resistant	10	0.3	30	30
E. coli Waksman	Unmodified	0.1	0.1	3	3
	S-resistant	1,000	0.3	1,000	1,000
	F-resistant	1.0	1.0	10	10
S. aureus 209	Unmodified	0.1	0.1	3	1
	S-resistant	1.0	0.3	30	30
	F-resistant	1.0	1.0	30	10
P. aeruginosa	Unmodified	3	3	10	10
	S-resistant	1,000	300	1,000	1,000
	F-resistant	1,000	1,000	1,000	1,000

The preceding results gave the first definite indication that preparations of "F" varied as to their antibiotic constitution. Accordingly, for a group of "F"

preparations obtained by slightly different procedures, activities were determined by the afore-mentioned plate assay with *B. subtilis* and *E. coli* against a streptomycin standard. The *E. coli* and *B. subtilis* values and their ratios are given in table 4. Whereas most ratios approximated 1.00, five preparations gave low ratios.

Fractionation of antibiotic activity of "F." As has been shown, preparations of "F" resemble streptomycin in their bacterial spectra (notable exception, Bodenheimer organism). This similarity was borne out in chemical and phys-

TABLE 4

E. coli : B. subtilis ratios of "F" preparations

NO.	E. COLI, u/MG	B. SUBTILIS, u/MG	E. COLI:B. SUBTILIS RATIO
1	684	579	1.18
2	512	450	1.14
3	453	424	1.07
4	406	428	0.95
5	438	456	0.96
6	250, 203	500	0.50, 0.41
7	482	458	1.05
8	380	380	1.00
9	470	540	0.87
10	340, 460	716	0.48, 0.64
11	488	470	1.04
12	528	469	1.12
13	416	388	1.07
14	304	369	0.82
15	249	290	0.86
16	279, 284	555	0.50, 0.51
17	126, 128	234	0.54, 0.55
18	418, 371	583	0.72, 0.64
19	851	756	1.12
20	816	821	0.99
21	715	766	0.93
22	503	635	0.79
23	327	377	0.86

ical tests. Both antibiotics gave a positive Sakaguchi reaction, yielded maltol (Schenck and Spielman, 1945) on alkaline hydrolysis, gave positive oxidized nitroprusside tests, showed only end absorption in the ultraviolet range of the spectrophotometer, and could be processed from culture filtrates in the same manner. However, lack of correspondence of the "F" preparations to streptomycin was indicated by the cysteine inactivation, iodine regeneration experiments, *E. coli:B.subtilis* ratios, and by the susceptibility of streptomycin-resistant cultures to some preparations of "F." These data suggested that antibiotic "F" was a mixture of biologically active substances, one of which might be streptomycin or streptomycinlike. The variation in similarities and dissimilarities of streptomycin and different "F" preparations seemed to indicate variation in the biologically active components of "F."

In view of the foregoing considerations it was decided to investigate "F" preparations for mixtures of antibiotics. First, streptomycin-resistant "F"-susceptible, and streptomycin-susceptible "F"-resistant, bacteria were sought from a natural source (soil) to provide means for differentiation. About 20 bacteria were readily isolated that were resistant to 1,000 u per ml of streptomycin and susceptible to 1 to 100 u per ml of "F" by the agar plate streak method. Attempts to find bacteria resistant to appreciable quantities of "F" were unsuccessful.

By the use of these recently isolated test bacteria and also the Bodenheimer organism, spectrum analyses were conducted on preparations of "F" and on fractions that were obtained by chromatographic treatment (Brockman alumina column). The data are given in table 5. Streptomycin and streptothricin were included for comparison. Each antibiotic was used at levels of 0.3, 1.0, 3, 10, 30, 100, 300, and 1,000 u per ml.

TABLE 5

Bacterial spectra of "F" preparations

Figures represent u per ml (*B. subtilis* assay) of antibiotic just preventing growth of test organism. Tryptose agar used. Incubation 24 hours at 28 C. Streak agar plate technique employed

ANTIBIOTIC	U/MG	TEST ORGANISM										
		No. 3	No. 4	No. 6	No. 10	No. 12	No. 14	No. 15	No. 17	No. 21	No. 28	Bodenheimer
Streptomycin	440	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000	1,000	>1,000	>1,000	>1,000
Streptothricin	270	10	30	3	30	3	1	100	1	>100	30	30
F no. 1	705	30	30	3	30	10	3	30	10	100	30	100
F no. 2	617	10	30	10	30	10	10	30	3	100	100	30
F no. 3	579	10	30	3	10	3	1	30	3	300	10	30
F no. 4*	800	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000	100	>1,000	>1,000
F no. 5*	250	1	3	1	1	1	1	3	1	100	3	10
F no. 6*	150	1	1	1	1	3	1	3	1	100	3	30

* Fractions from chromatographic column.

Table 5 indicates some spectrum differences among original preparations of "F" (nos. 1, 2, and 3) suggesting that different lots vary biologically. In general, however, their spectra correspond closely to that for streptothricin.

When an "F" preparation was fractionated chromatographically, antibiotic fractions of a type corresponding to no. 4 were obtained from the first elution. This material not only resembled streptomycin biologically, but also by such chemical tests as quantitative Sakaguchi determination, complete inactivation with ketone reagents, maltol formation on alkaline hydrolysis, specific rotation, and chemical analysis. Following elution of the streptomycinlike material, a second antibiotic material was obtained of a more streptothricinlike character (nos. 5 and 6).[1]

Since the foregoing work strongly indicated that "F" preparations were composed of at least two antibiotic substances, efforts were directed to evolving a procedure for assaying one antibiotic in a mixture. The isolated streptomycin-resistant "F"-susceptible bacteria were examined in an attempt to obtain a

[1] Richardson, E. R., Trussell, P. C., and Grant, G. A., unpublished data.

suitable test organism. Although some gave favorable results, the Bodenheimer organism was most suitable. With this organism there was a linear relation between the diameter of the zone of inhibition and the logarithm of the dosage between the limits of 5 and 40 u per ml of streptothricin.

Streptomycin alone did not inhibit the Bodenheimer organism at concentrations of 1,000 u per ml, but it enhanced the inhibitory effect of streptothricin when both were present. Gradational increases in inhibition of the test organism were observed when streptothricin concentration was kept constant while that of streptomycin was increased from 100 to 400 u per ml; but from 400 to 1,000 u per ml of streptomycin did not further increase the inhibition. Accordingly, all dilutions of the streptothricin standard as well as those of "F" were made to contain 400 u per ml of streptomycin. Good reproducibility was thus obtained in assays of "F" preparations for streptothricinlike activity, the results of which

TABLE 6

Percentage of streptothricinlike material in "F" preparations and fractions

PREP'N NO.	POTENCY, U/MG		
	B. subtilis	Bodenheimer	% streptothricinlike
1	438	60, 61, 74, 74	14, 14, 17, 17
2	569	10	<2
3	208	54	27
4	265	180, 175	68
5	136	35	26
6	583	332, 321	55
7	705	440	62
8D	989	<45	<4
8E	902	<15	<2
8F	778	<30	<3
8G	590	154	26
8H	570	200	35
8I	306	88	28

are given in table 6. This shows that different preparations may vary from <2 to 68 per cent streptothricinlike material. The no. 8 series consists of consecutive fractions from a chromatographic column. Appreciable amounts of the streptothricinlike material are present in the last three fractions, and relatively little in the first three.

Rate of elaboration of antibiotic components of "F." Six shake flask cultures (150 ml) were assayed against *B. subtilis* and the Bodenheimer organism (cup assay) at 4, 5, and 9 days of incubation, to determine total antibiotic activity and streptothricinlike activity, respectively. The data appear in table 7. The Bodenheimer-active fraction was produced in much smaller amounts than the streptomycinlike fraction and reached its peak about the fifth day. The streptomycinlike fraction was produced chiefly after the fourth day, reaching a maximum at the ninth day. This difference in rate of formation is shown more clearly by the ratio of Bodenheimer organism activity to *B. subtilis* activity.

Intravenous toxicity. Preliminary tests were made on a number of column fractions: fraction I, streptomycinlike (not inhibiting Bodenheimer organism at 1,000 u per ml); and fractions II, III, and IV, containing 55, 30, and 65 per cent of streptothricinlike activity, respectively.

TABLE 7

Rate of formation of antibiotic components of "F" during fermentation

FERMENT NO.	4 DAYS			5 DAYS			9 DAYS		
	B. SUBT., u/ML	BODEN., u/ML	RATIO*	B. SUBT., u/ML	BODEN., u/ML	RATIO	B. SUBT., u/ML	BODEN., u/ML	RATIO
1	75	60	0.80	204	107	0.53	370	64	0.17
2	40	43	1.07	128	72	0.56	335	70	0.21
3	46	54	1.17	200	85	0.43	330	64	0.19
4	93	62	0.67	285	100	0.35	390	71	0.18
5	112	72	0.64	322	102	0.32	388	71	0.18
6	86	56	0.65	276	101	0.36	381	62	0.16
Average.....			0.83			0.43			0.18

* Ratio, Bodenheimer organism activity:*B. subtilis* activity.

TABLE 8

Toxicity of "F" preparations

FRACTION NO.	NO. OF MICE	TOTAL UNITS INJECTED INTRAVENOUSLY	PURITY OF SAMPLE UNITS PER MG	STREPTOMYCINLIKE UNITS INJECTED	STREPTOTHRICINLIKE UNITS INJECTED	IMMEDIATE DEATHS	DELAYED DEATHS									% Dead at end of 12 days
							Days									
							1	2	3	4	5	6	7	8	9 to 12	
I	10	5,000	775	5,000	0	10										100
	10	3,000	775	3,000	0	4	0	0	0	0	0	0	0	0	0	40
	10	1,500	775	1,500	0	0	0	0	0	0	0	0	0	0	0	0
	10	750	775	750	0	0	0	0	0	0	0	0	0	0	0	0
II	10	1,000	530	450	550	0	0	0	5	4	0	0	0	0	0	90
	5	500	530	225	275	0	0	1	0	0	1	0	0	0	0	40
III	8	3,042	640	2,129	913	8										100
	8	1,521	640	1,065	456	0	0	0	1	0	0	0	0	0	0	12
IV	8	3,000	705	1,050	1,950	3	0	0	0	0	1	1	0	1	0	75
	8	1,500	705	525	975	0	0	0	0	1	0	0	0	0	0	12
	8	750	705	263	487	0	0	0	0	0	1	0	0	0	0	12

The results are summarized in table 8. The toxicity of fraction I is similar to that of streptomycin, the LD_{50} being 3,000 units. (This streptomycinlike fraction injected subcutaneously, 2,400 units daily, into each of 10 mice for 6 days produced no apparent ill effect after 10 days.) Fractions II, III, and IV, containing streptothricinlike material, showed delayed toxicity and were more toxic than fraction I.

More conclusive toxicity tests of the streptothricinlike activity await further separation of the antibiotic components.

In vivo activity. Protective properties of "F" compared to streptomycin are indicated in table 9.

Mice injected with a single intraperitoneal dose of 500 units of a preparation of "F" (65 per cent streptothricinlike) survived longer against a lethal dose of *Salmonella suipestifer* intraperitoneally than did mice receiving a similar dose of streptomycin. No significant protective action was noted when 1,000 units of "F" were given orally, whereas oral streptomycin had a slight effect. A single subcutaneous 500-unit dose of "F" extended more protection to mice against a lethal dose of *Salmonella schottmuelleri* intraperitoneally than did a similar dose of streptomycin.

TABLE 9

In vivo activity

NO. MICE	ANTIBIOTIC	SINGLE DOSE UNITS	ROUTE*	ORGANISM	% SURVIVAL		
					3 days	10 days	30 days
10	"F"	500	I.P.	*S. suipestifer*	90	0	0
10	Streptomycin	500	I.P.	*S. suipestifer*	33	0	0
10	None	0		*S. suipestifer*	0	0	0
10	"F"	1,000	Oral	*S. suipestifer*	0	0	0
10	Streptomycin	1,000	Oral	*S. suipestifer*	10	0	0
10	None	0		*S. suipestifer*	0	0	0
10	"F"	500	S.C.	*S. schottmuelleri*	100	90	70
10	Streptomycin	500	S.C.	*S. schottmuelleri*	100	60	20
10	None	0	S.C.	*S. schottmuelleri*	40	0	0

* I.P. = intraperitoneal injection; S.C. = subcutaneous injection.

ACKNOWLEDGMENTS

The authors are indebted to Mr. A. Barol, Wyeth Institute of Applied Biochemistry, for supplying the culture of the Bodenheimer organism, and to Dr. S. A. Waksman, New Jersey Agricultural Experiment Station, Rutgers University, for cultures of *S. lavendulae* and *S. griseus*; they also express their appreciation to Mr. Ivan Brown, Miss Joyce Wilson, and Miss June Perry for valuable technical assistance.

DISCUSSION AND SUMMARY

According to Waksman's classification key, the *Streptomyces* employed in this study is distinct from either *S. griseus* or *S. lavendulae*, as indicated from a determinative study of the three streptomycetes on 36 diagnostic media that included 7 carbon and 10 nitrogen sources. Purity of the isolate was assured by replating and by bacterial spectrum studies on culture filtrates from individual colonies of the mother culture.

Accordingly, the premise seems acceptable that the isolate is an organism not previously described and that it elaborates a mixture of antibiotics. Whether this mixture is composed of only two separate active fractions is as yet uncertain. Evidence favors the identity of one fraction with streptomycin. All that may be presumed of the second fraction at present is that it is like streptothricin.

Preparations containing the streptothricinlike component are toxic. The antibiotic mixture produced by this new isolate of *Streptomyces* has been found to possess *in vivo* activity.

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