sions of other tissues correspond to those of human rheumatic fever and its related diseases; and third, some or most of the clinical and laboratory findings of the human disease are reproduced in animals. Until such time they might be called either "protein carditis," or "rheumatic-like carditis." If, however, one were to hypothecate that human rheumatic fever is the result of parenteral contact with foreign protein to which the tissues have been sensitized, one might expect that hypothesis to be reasonably compatible with the known relation of the streptococcus to the disease, for it would indeed be difficult to find a source better than the streptococcus of foreign protein which customarily, frequently, and intermittently gains access to the more intimate tissues and vascular system of man.

Summary. Acute rheumatic-like heart lesions have been produced in mice by parenteral injection of egg white on repeated occasions. Minimal lesions may also occur in untreated mice, possibly as a result of spontaneous sensitization and shocking by natural contact with protein. There was no apparent relation between the severity of clinical anaphylaxis and the severity of the pathologic changes in the heart.

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On Some Biological Characteristics of Streptomycin B.

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Fried and Titus[1] recently described the isolation, from streptomycin concentrates, of a new entity which they called streptomycin B. Chromatographic fractions described by these authors were made available for biological studies. The materials used for the present work were rich in streptomycin B and from Craig countercurrent distribution data[2] were estimated to contain not more than 10-15%, by weight, of streptomycins other than B. For purposes of comparison, studies with a highly purified preparation of streptomycin, free of streptomycin B, were included in the investigations. In this paper the term "streptomycin" will refer to the preparation containing no streptomycin B, while "streptomycin B" will refer to the above described chromatographic fractions rich in this latter material.

I. *In vitro studies.* The minimal inhibiting concentrations (M.I.C.) of streptomycin and streptomycin B for 5 strains or species of bacteria were determined in yeast beef broth, and for 2 species of mycobacteria in Kirchner's medium modified* to contain albumin and Tween 80 as in media described by Dubos and Davis.[3] The M.I.C. values thus determined are shown in Table I.

The importance of the type of culture medium used for such studies is clearly shown by the comparisons in Table II where addi-

[1] Fried, J., and Titus, E., *J. Biol. Chem.*, 1947, **168**, 391.

[2] Titus, E., and Fried, J., *J. Biol. Chem.*, 1947, **168**, 393.

* Modified Kirchner's synthetic medium for growth of *M. tuberculosis:*

Na_2HPO_4	3 g
KH_2PO_4	4 g
$MgSO_4$	0.6 g
Sodium citrate	2.5 g
Iron ammonium citrate	0.05 g
Asparagin	5.0 g
Glycerine	20 ml
Distilled water	1000 ml
Tween 80	0.05%

Autoclave at 15 pounds for 20 minutes.

Solution of human serum albumin sterilized by filtration added to 0.1%.

[3] Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, **83**, 409.

TABLE I.
Comparative *in vitro* Activities of Streptomycin B and Streptomycin.

Test organism	M.I.C. in yeast beef broth*		Ratio Streptomycin B / Streptomycin
	Streptomycin-trihydrochloride γ/ml	Streptomycin B-hydrochloride† γ/ml	
Eberthella typhosa (Hobby)	9.94	12.2	1.23
Salmonella schottmulleri	8.42	13.5	1.61
Mycobacterium tuberculosis (H37Rv)*	1.01	3.73	3.70
Klebsiella pneumoniae (ATCC 9997)	1.53	5.70	3.73
*Mycobacterium smegmatis**	1.38	6.80	4.92
Staphylococcus aureus (Osgood)	1.50	7.80	5.20
Staphylococcus aureus (209-P)	1.35	9.55	7.06

* The two strains of mycobacteria were tested in a modified Kirchner's synthetic medium rather than in yeast beef broth.

† Several streptomycin B preparations all of approximately equivalent compositions were used for these studies. The figures given are averages of all data obtained.

TABLE II.
Effect of Culture Media on Relative Activities of Streptomycin and Streptomycin B Against Several Organisms.

Test organism	Material tested	M.I.C. in Yeast beef broth γ/ml	M.I.C. in Tryptone broth γ/ml	Ratio of M.I.C.'s Yeast beef/Tryptone	"Interference ratio"
K. pneumoniae	Streptomycin	1.53	0.0442	34.6	0.82
	Streptomycin B*	5.70	0.200	28.5	
	Streptomycin B/Streptomycin	3.73	4.53	—	—
E. typhosa (Hobby)	Streptomycin	9.94	0.188	52.8	1.15
	Streptomycin B*	12.2	0.200	61.0	
	Streptomycin B/Streptomycin	1.23	1.06	—	—
S. schottmulleri	Streptomycin	8.42	0.188	44.8	1.31
	Streptomycin B*	13.5	0.231	58.6	
	Streptomycin B/Streptomycin	1.61	1.23	—	—

* Several streptomycin B preparations all of approximately equivalent compositions were used for these studies. The figures given are averages of all data obtained.

tional data gathered in 0.75% tryptone broth, pH 8.5, are listed together with data taken from Table I. It perhaps should be emphasized at this point that the M.I.C. values listed in these tables are given in actual weight of material required to inhibit, and not in terms of streptomycin base. Since the streptomycin B preparations used were not entirely free of streptomycin, these figures will eventually have to be modified, and differences from streptomycin will undoubtedly be even greater than here shown.

It will be noted that in the various culture media used the M.I.C. values for streptomycin were always lower than those of streptomycin B. However, the manner in which a given culture medium affected the activity of either antibiotic depended on the test organism. Thus, examination of Table II reveals that for *K. pneumoniae,* 34.6 times as much streptomycin was required to inhibit growth in yeast beef broth as in 0.75% tryptone broth, pH 8.5. Of streptomycin B, 28.5 times as much antibiotic was required in yeast beef broth as in tryptone broth. The ratio 28.5/34.6 = 0.82 we have called an

"interference ratio" and indicates that for this test organism yeast beef broth in comparison to tryptone broth interfered more with the action of streptomycin than with that of streptomycin B. On the other hand, the reverse was true when *E. typhosa* or *S. schottmülleri* were used as test organisms since the "interference ratios" for these 2 organisms were 1.18 and 1.31 respectively.

Since *K. pneumoniae* showed relatively greater sensitivity to streptomycin B as the culture medium was changed from a simple tryptone broth to a richer yeast beef broth, the possibility exists that the relative sensitivity of this organism towards streptomycin B *in vivo* might be even greater than *in vitro*. If this were so, one might expect *E. typhosa* and *S. schottmülleri* to show relatively less sensitivity to streptomycin B *in vivo*. Insufficient quantities of this material prevented testing it against all 3 organisms *in vivo*, but tests with *S. schottmülleri* in mice (see section on *in vivo* studies) lends evidence in favor of this hypothesis since the *in vitro* action of streptomycin was 1.2-1.6 times as great as that of streptomycin B for this organism (Table II) while *in vivo* the former was 3 times as active as the latter in experimental infection in mice.

It is of interest to note that at a recent conference on antibiotics, Hobby and Lenert[4] reported that certain residues, obtained in the preparation of crystalline salts of streptomycin, were from 2 to 5 times more active than highly purified streptomycin sulfate against a number of strains of *E. typhosa*. One of these strains was kindly supplied us by Dr. Hobby and its behavior towards streptomycin B was determined. The results of the tests with this organism are included in Tables I and II. It is to be assumed that Hobby and Lenert's[4] conclusions were drawn on the basis of activities given in streptomycin units as measured by a standard organism. When the streptomycin and streptomycin B used in the present studies were assayed against a streptomycin standard with *K. pneumoniae* as the test organism, the streptomycin employed had an activity of 650 u/mg and the various streptomycin B preparations had activities between 140 and 200 u/mg.† On this basis *E. typhosa* would require ca. 6 u of streptomycin to be inhibited in yeast beef broth, and ca. 1.5 u of streptomycin B. Hence, on a streptomycin unit basis, streptomycin B would appear to be more active than streptomycin against *E. typhosa*. This would also hold for the strain of *S. schottmülleri*,‡ as well as for a strain of *S. enteritidis*.§

It is of further interest to note that streptomycin B is not precipitated as a calcium chloride double salt[5] and, therefore, may well

[4] Hobby, G. L., and Lenert, T. B., The Action of Streptomycin *in vitro*. Conference on Antibiotic Research; Antibiotic Study Section of the National Institute of Health, Washington, D.C., Jan. 31-Feb. 1, 1947.

† With regard to the assignment of bioactivities, on the basis of streptomycin units, to antibiotics other than streptomycin itself, both the test organism and the test medium are of prime importance. Streptomycin bioassay procedures have been described in various laboratories using as the test organism any of a number of species of organisms and various test media. Even where a common streptomycin standard has been used, the units measured by the various procedures are equivalent only so long as streptomycin alone is present. If, on the other hand, an antibiotic other than streptomycin is to be bioassayed, the common streptomycin standard is of little use since the various test organisms may respond to the new antibiotic in entirely different fashions. This is well exemplified by comparing the data for *K. pneumoniae* and *Staph. aureus* (Osgood) in Table I. These two species are equally sensitive to streptomycin in yeast beef broth, but they differ significantly in their respective sensitivities to streptomycin B. Hence, the streptomycin unit loses its meaning when applied to antibiotics other than streptomycin.

‡ The authors wish to thank Mr. Otto Graessle of the Merck Institute for Therapeutic Research for the *S. schottmulleri* culture used here.

§ The strain of *S. enteritidis* referred to was tested in 0.75% tryptone broth, pH 8.5, but not in yeast beef broth, and therefore was not reported in Table I. In tryptone broth the M.I.C. of streptomycin for this organism was 0.079 γ/ml as compared to 0.187 γ/ml of streptomycin B, giving a streptomycin B/streptomycin ratio of 2.36.

[5] Fried, J., personal communication.

have been present in residues described by Hobby.[4] There is, therefore, reason to believe that the activity in residues referred to by Hobby may be due, at least in part, to the streptomycin B described by Fried and Titus.[1]

Effect of streptomycin B on an organism resistant to streptomycin. Unless special precautions are taken, even highly purified streptomycin preparations may contain some streptomycin B. Hence organisms made resistant to the usual streptomycin preparations may have been exposed also to streptomycin B and it would, therefore, not be surprising to find such organisms also resistant to the latter material. For the present work efforts were made to avoid such conditions in order to study whether development of resistance towards streptomycin was accompanied by resistance to streptomycin B. Hence, a freshly isolated strain of *E. coli,* highly sensitive to streptomycin, was made 25 times more resistant than originally by exposure to sublethal amounts of streptomycin free of streptomycin B. It was found that this organism had become similarly more resistant to streptomycin B. The lack of a preparation of streptomycin B entirely free of streptomycin, *per se,* prevented testing the reverse process, *i.e.* making an organism resistant to B and then testing its sensitivity to streptomycin free of streptomycin B.

II. *In vivo studies. A. Toxicity of streptomycin B in mice.* The intravenous toxicity of streptomycin B was compared with that of streptomycin in Swiss albino mice. Weight for weight the toxicities of the 2 materials were found to be very similar. Thus, a dose of ca. 5 mg/mouse (ca. 250 mg/kg) of either antibiotic caused severe shock and occasional immediate deaths. Higher doses (*e.g.* 7.0 mg/mouse) invariably caused immediate death while lower doses caused no deaths if the time taken for injection was at least 15 seconds. The appearance of mice in shock caused by either material was entirely similar and in neither case did any deaths or other signs or symptoms occur during a 21-day period following injection. Mouse weights and food intake data gathered during this period were normal and autopsies at the end of this interval showed no pathological changes.

B. Therapeutic action of streptomycin B. 1. In experimental tuberculosis in mice. Procedure. In experimental tuberculosis in mice the strain of mouse used is extremely important.[6] Studies (to be described elsewhere) as to the choice of mouse strain as well as the strain of *M. tuberculosis* to be used for such investigations as the present led to the use of a bovine strain of *M. tuberculosis* (Ravanel) and a strain of albino mouse known as the CF1 (Carworth Farms).

a. Inoculum. The Ravanel strain of *M. tuberculosis* was grown in a modified Kirchner's synthetic medium (described above). Inocula were usually prepared from a 5-day culture diluted to match the turbidity of a McFarland turbidity standard consisting of 0.0125 ml of 1% barium chloride solution plus 9.99 ml of 1% sulfuric acid. As a rule this meant diluting a 5-day culture 1/10 with physiological saline.

CF1 mice, weighing 16-18 g, were given 0.5 ml of the appropriately diluted culture intravenously. The dose per mouse was roughly equivalent to 0.6 mg of organisms (moist weight).

b. Treatment. Subcutaneous administration of streptomycin or streptomycin B was begun within an hour following the time of infection. A schedule of three 0.1 ml injections per day (morning, noon, and late afternoon) for 21 days was followed in the experiment reported here.

Results. In Table III is shown the comparative therapeutic action of streptomycin and streptomycin B. The antibiotic preparations used were similar to those described in the *in vitro* studies. Because of the small amounts of streptomycin B available, it was possible to have only 5 mice per dose in this group.

It will be recalled (Table I) that when tested *in vitro,* streptomycin was 3.7 times as active as streptomycin B (weight for weight) against *M. tuberculosis* H37Rv. It is evident from the data in Table III that a roughly similar relationship exists for the *in vivo*

[6] Pierce, C., Dubos, R. J., and Middlebrook, G., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 173.

TABLE III.
Comparative Therapeutic Action of Streptomycin B and Streptomycin in Experimental Tuberculosis in Mice.

Antibiotic	Dose mg/kg/day	Dose u/kg/day*	No. of days treated	Deaths within 30 days	Avg survival time,† days
Streptomycin	49.45	32,100	21	0/10	
	12.36	8,025	21	9/10	25.0
	3.09	2,006	21	10/10	21.7
Streptomycin B	155.47	30,220	21	0/5	
	38.87	7,550	21	4/5	26.8
Untreated Controls				10/10	20.6

* The streptomycin used had an activity of 650 u/mg; that of the streptomycin B would be assigned on activity of 194 u/mg on the basis of assays with *K. pneumoniae* against a streptomycin standard.

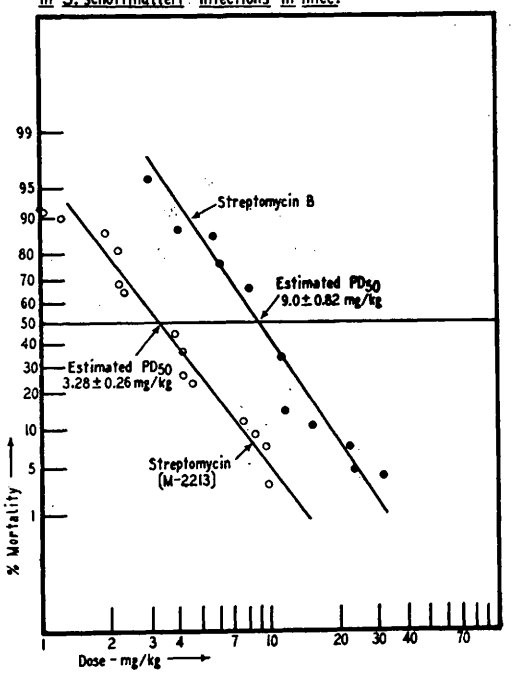
† For purposes of calculation, mice surviving on the 30th day were assumed to have died on the 31st.

actions of these 2 antibiotics against the Ravanel strain in mice. Thus, using small numbers of mice complete protection for 30 days‖ was brought about by approximately ⅓ as much streptomycin as was required of streptomycin B. Since this is roughly the inverse ratio of the relative potencies which these 2 materials would have on the basis of units as measured by *K. pneumoniae* (650 u/mg as compared with 140-200 u/mg for streptomycin B) it can be seen that on a *unit basis* the 2 antibiotics would appear to be approximately equal in therapeutic action in experimental tuberculosis in mice. Final conclusions, of course, are not yet permissible since of necessity only small groups of mice were used in these studies and the steps in dosage range were 4-fold.

2. Comparative action of streptomycin and streptomycin B in experimental Salmonella schottmulleri infections in mice. Procedure. Sixteen-hour beef heart broth cultures diluted in 5% mucin were used for inoculating Swiss albino mice intraperitoneally. Mice to be treated received 1 ml, intraperitoneally, of a 1×10^{-7} dilution of a culture while control mice received 1 ml of dilutions ranging from 1×10^{-6}, through 10^{-9}. Plate counts of the cultures indicated that one organism per mouse proved fatal. Thus, in composite results of 5 tests the 10^{-7} dilution on the average contained 85 organisms per ml and killed 50/50 mice; 10^{-8} dilution (average 8.5 organisms per ml) killed 46/50 mice and 10^{-9} dilution (average 0.85 organisms per ml) killed 23/50 mice. Hence, the mice to be

‖ The dose levels used were adjusted to make comparisons possible at 30 days, but not high enough to prevent infection entirely. In other studies (to be described elsewhere) it was found that even doses of streptomycin 10 times as great as used here would not prevent eventual deaths of mice infected with tuberculosis.

— FIGURE I —
Comparative action of Streptomycin and Streptomycin-B in S. schottmulleri infections in mice.



treated received approximately 100 LD_{50}.

Treatment. Streptomycin and streptomycin B, in aqueous solutions, were administered in a single dose, 0.5 ml subcutaneously, within an hour after infection.

Results. Composite results of 3 tests with streptomycin and streptomycin B are shown in Fig. 1. In each test each therapeutic agent was tested at 4-dose levels, with 10 mice per level. Results, therefore, were calculated on the basis of response of 120 mice for each compound. For calculating the PD_{50} (dose protecting 50% of the mice), the composite data was first treated according to the method of Reed and Muench[7] and then plotted on probability log paper. Here the estimated PD_{50} was determined graphically and the standard error calculated by the method described by Miller and Tainter.[8]

The estimated PD_{50} for streptomycin was found to be 3.28 ± 0.26 mg/kg while that for streptomycin B was 9.0 ± 0.82 mg/kg. Hence, on a weight basis streptomycin was therapeutically approximately 3 times as active as streptomycin B. If one were to convert these weights to units as assayed with *K. pneumoniae* against a standard, the PD_{50} of streptomycin (at 650 u/mg) would be 2120 u/kg, and streptomycin B (at 170 u/mg) would be 1530 u/kg. Hence, on the unit basis now in use, streptomycin B would again appear to be more active than streptomycin.

It will be recalled, from the *in vitro* studies, (Table II) that in tryptone broth streptomycin was 1.23 times as active as streptomycin B, on a weight for weight basis, and 1.61 times as active as the latter in yeast beef broth against the same strain of *S. schottmulleri,* while it has now been shown that *in vivo,* streptomycin was ca. 3 times as active as streptomycin B. This may, of course, be due to differences in disposition of the 2 substances in the body, or perhaps, as suggested above, to differential effects of the body fluids on the activities of these 2 antibiotics. Final answer to this question must await, at least in part, on absorption-excretion studies with streptomycin B.

Summary. Streptomycin B, an antibiotic closely related to streptomycin, and separated from the latter by chromatography, has been studied *in vitro* against 8 strains or species of bacteria and in each case shown to be less active, on a weight basis, than streptomycin *per se.*

Toxicity studies in mice indicated that streptomycin B and streptomycin have approximately equal LD_{50} values on a weight basis.

In experimental tuberculosis infections in mice, streptomycin B was about ⅓ as active (again weight for weight) as was streptomycin, but was approximately equally active as the latter on the basis of presently accepted units.

In experimental *S. schottmülleri* infections in mice, streptomycin B again had ⅓ the activity of streptomycin on a weight basis, but would appear to be more active than the latter if comparison was made on a unit basis.

The authors wish to acknowledge the technical assistance of Mr. D. P. Canales in carrying out the experiments described in this paper.

[7] Reed, L. J., and Muench, H., *Am. J. Hygiene,* 1938, **27**, 493.

[8] Miller, L. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 261.