THE EFFECT OF AUREOMYCIN AGAINST *BACTERIUM TULARENSE* [1]

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The wide antibiotic spectrum of aureomycin prompted exploration for possible activity against *B. tularense.* Its evidently low toxicity and its absorption in active form when administered orally would be decided advantages should it prove to be effective in tularemia.

EXPERIMENTAL STUDIES

The test organism. Studies were undertaken using a virulent strain of *B. tularense* recently recovered from a patient who died of tularemic meningitis (1). The organism was agglutinated by known tularemic serum to full titer. It failed to grow on blood agar but grew well on solid medium containing 8% fresh rabbit blood, 1% dextrose, and 0.1% l-cystine in beef heart infusion agar base, prepared according to the method of Francis (2). Early cultures were preserved at −20° C. or 4° C. Fresh 24 to 48 hour transplants on blood dextrose cystine agar were used in all experiments, representing the second to seventh transfers from the original culture. The purity of each culture used was checked microscopically and on blood agar plates.

1. *Inhibition of* B. tularense *by aureomycin in culture medium*

Method: Fresh medium was prepared by adding 8% rabbit blood to dextrose cystine beef heart infusion agar at 60° C. For comparison with control medium, aureomycin-HCl [2] from 20 mg. vials was dissolved in distilled water and introduced into flasks of this medium to yield final concentrations of 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 micrograms per ml. Exposure of the drug to 60° C. was momentary. The medium was quickly tubed, slanted, and hardened in the icebox. The elapsed time between dissolving the aureomycin and inoculation of the slants was five to six hours. The final pH of the medium was 6.8, and was not changed detectably by addition of aureomycin-HCl in concentration of 64 micrograms per ml.

The effectiveness of aureomycin in this medium against *B. tularense* and *Staph. aureus* was tested. The staphylococcus used was inhibited by 1.5 but not by 0.75 micrograms of aureomycin per ml. in tryptose phosphate broth of pH 7.2 at 24 hours incubation. Turbid suspensions in broth were made from 26 hour blood dextrose cystine agar cultures of each organism. Slants containing 0 to 8 micrograms aureomycin per ml. were inoculated with 1 loopful of *Staph. aureus* or 3 loopfuls of *B. tularense* and incubated at 37° C.

Results: Growth of *B. tularense* did not become apparent on the control medium until 24 hours, at which time 0.5 microgram of aureomycin per ml. caused complete inhibition, while *Staph. aureus* required 1 microgram per ml. for inhibition (Table I). These early results are difficult to interpret because *B. tularense* grows more slowly. After 36 hours the concentrations of aureomycin required to inhibit the two organisms were comparable, approximately doubling each 24 hours until 96 hours when growth of both was observed with 8 micrograms per ml. Earlier experiments showed that neither organism grew on slants containing 16 or more micrograms per ml. after incubation for one week.

TABLE I

INHIBITION OF B. TULARENSE AND STAPH. AUREUS BY AUREOMYCIN IN BLOOD DEXTROSE CYSTINE AGAR SLANTS

ORGANISM	HOURS INCUBATION AT 37° C.					
	12	24	36	48	67	96
	CONC. OF AUREOMYCIN CAUSING COMPLETE INHIBITION, γ/ml					
STAPH. AUREUS*	0.5	1.0	2.0	2.0	4.0	>8.0
B. TULARENSE	?	0.5	2.0	2.0	4.0	>8.0

* THIS STRAIN IS INHIBITED BY AUREOMYCIN 1.5 MICROGRAMS PER ML IN TRYPTOSE PHOSPHATE BROTH AT 24 HOURS INCUBATION.

Aureomycin in concentrations of 8 micrograms or less per ml. thus inhibited but did not kill *B. tularense* and *Staph. aureus* inoculated on blood dextrose cystine agar slants. The progressive

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[2] The aureomycin used in these studies was supplied through the courtesy of Lederle Laboratories Division, American Cyanamid Co.

daily increase in the concentration required for inhibition is best explained by deterioration of the drug on incubation at 37° C., as shown by Paine *et al.* (3) and Bliss and Chandler (4). The strain of *B. tularense* tested was inhibited by concentrations comparable to those required for inhibition of a sensitive strain of *Staph. aureus.*

2. *Effect of incubating* B. tularense *with aureomycin in broth*

Method: A turbid suspension of *B. tularense* with a density of 4 on the McFarland scale (5) was prepared in tryptose phosphate broth from a 41 hour blood dextrose cystine agar culture. Aliquots of this suspension were added to equal volumes of broth, of aureomycin-HCl 100 micrograms per ml. in distilled water, and aureomycin 1000 micrograms per ml. in distilled water yielding final aureomycin concentrations of 0, 50, and 500 micrograms per ml. The initial pH of the broth was 7.2 and was not changed detectably by addition of aureomycin 50 micrograms per ml. It was reduced only to 7.05 by addition of 500 micrograms per ml. This is well within the pH range for maximal growth of *B. tularense.* The suspensions were incubated at 37° C. for four hours, after which tenfold serial dilutions in broth were made.

Results: Inocula of 0.1 ml. of dilutions 10^{-1} to 10^{-10} were injected intraperitoneally into groups of four or five mice (Table II). All mice receiving dilutions 10^{-1} to 10^{-6} inclusive died. The control suspension incubated with broth had an LD_{50} of 10^{-9} by the method of Reed and Muench (6). When incubated with 50 micrograms of aureomycin per ml. the LD_{50} was $10^{-8.8}$ and with 500 micrograms per ml. it was $10^{-7.2}$.

Slants of blood dextrose cystine agar were also inoculated with three loopfuls of 10^{-1} and 10^{-8} dilutions prepared from the three suspensions after incubation as described above. When incubated three days at 37° C. the slants from both dilutions of the control broth suspension and from the 10^{-1} dilution of the suspension containing aureomycin 50 micrograms per ml. showed a heavy confluent growth of *B. tularense.* The 10^{-8} dilution of the suspension containing aureomycin 50 micrograms per ml. and the 10^{-1} dilution of the suspension containing 500 micrograms per ml. yielded innumerable separate colonies. The 10^{-8} dilution of the latter gave only 10–12 small colonies. Although after one week 65 colonies were visible, growth was still strikingly reduced as compared with cultures from the other suspensions.

It is evident that incubating a suspension of *B. tularense* for four hours at 37° C. in the presence of aureomycin 500 micrograms per ml. killed an appreciable number of organisms. Questionable reduction of viable bacteria occurred in the presence of 50 micrograms per ml.

TABLE II

EFFECT OF INCUBATING A BROTH SUSPENSION OF B. TULARENSE WITH AUREOMYCIN (4 HOURS AT 37°C) UPON INFECTIVITY FOR MICE

CONCENTRATION OF AUREOMYCIN γ/ML	DILUTION INOCULATED[1] (0.1 ML I-P)					50% MORTALITY TITER
	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	
0	0/4[2]	0/4	0/4	2/4	4/4	10^{-9}
50	0/5	0/4	1/4	4/4	4/4	$10^{-8.8}$
500	0/5	3/5	5/5	3/5	5/5	$10^{-7.2}$

[1] ALL MICE INOCULATED WITH DILUTIONS 10^{-1} TO 10^{-6} DIED

[2] NUMBER OF MICE SURVIVING 14 DAYS / NUMBER INOCULATED

3. *Subcutaneous aureomycin therapy of experimental tularemia in mice*

Method: In therapeutic experiments standard suspensions of *B. tularense* were freshly prepared by emulsifying growth from a 24 to 48 hour blood dextrose cystine agar culture in sterile saline and adjusting to a density of two on the McFarland scale (5). All suspensions were cultured on blood agar for purity and yielded no growth. Tenfold serial dilutions in broth were made. Young adult white male mice were inoculated intraperitoneally with 0.1 ml. of various serial dilutions. Control mice and those to be treated were inoculated with the same dilution. Simultaneously the LD_{50} of the suspension was determined by titration in additional groups of mice. Aureomycin-HCl dissolved in sterile distilled water was administered subcutaneously to the mice designated for treatment. Mouse tularemia is apparently not contagious to cage mates except perhaps by cannibalism, which was prevented so far as possible by prompt removal of dead animals.

Procedure and Results: A pilot experiment was performed to determine the LD_{50} for mice of a standard suspension of *B. tularense.* Two of the higher serial dilutions were arbitrarily chosen for treatment (Table III). Inocula of 0.1 ml. of dilutions 10^{-4} to 10^{-9} inclusive were injected intraperitoneally into groups of four mice. Additional groups of four mice received the 10^{-7} and 10^{-8} dilutions. Treatment of the two latter groups with subcutaneous injections of aureomycin-HCl 0.5

TABLE III

PROTECTIVE EFFECT OF AUREOMYCIN ADMINISTERED SUBCUTANEOUSLY TO MICE INFECTED WITH SMALL INTRAPERITONEAL INOCULA OF B. TULARENSE

TREATMENT	DILUTION INOCULATED (0.1 ML I-P)				50% MORTALITY TITER
	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
NONE	0/4[1]	0/4	0/4	3/4	$10^{-8.7}$
15 MG/DAY S-C, BEGUN IMMEDIATELY, 5 DAYS	-	4/4 10+ MLD	4/4 1+ MLD	-	-

[1] NUMBER OF MICE SURVIVING 21 DAYS / NUMBER INOCULATED

mg. was begun immediately and continued three times daily at approximately eight hour intervals for five days, a total of 16 injections. (Each dose consisted of 0.25 ml. of a solution containing 2 mg. of aureomycin-HCl per ml. in distilled water.) All untreated mice inoculated with dilutions 10^{-4} to 10^{-8} inclusive and one receiving 10^{-9} died. The LD_{50} of the suspension was $10^{-8.7}$. All treated mice inoculated with the 10^{-7} and 10^{-8} dilutions survived 27 days after inoculation. It was evident that the inocula were small, about 10 and 1 MLD respectively, but 100% survival of the treated animals was noteworthy.

To investigate the possibility that *B. tularense* was harbored in the tissues of the surviving treated animals, two mice were killed on the 27th day after inoculation with the 10^{-7} dilution. The spleen of one was enlarged to at least three times normal size, dark red, and showed definite pale focal areas on section. The spleen of the other was enlarged to perhaps twice normal size but showed only questionable foci. No other gross changes were noted. Spleen impression smears stained by Gram's method were negative for *B. tularense*. The spleens were ground with sterile sand and emulsified in broth. Cultures yielded no growth on blood agar and blood dextrose cystine agar. Five mice were inoculated intraperitoneally with 0.1 ml. One died on the third day and one on the 12th, apparently neither of tularemia as shown by absence of typical autopsy findings and by negative spleen impression smears. The other three mice survived for 21 days. Accordingly, no evidence was obtained in this experiment to suggest that viable *B. tularense* persisted in the spleens of mice which survived as a result of aureomycin treatment.

To investigate the possible development of immunity in treated animals, the remaining six mice which had survived inoculation with the 10^{-7} and 10^{-8} dilutions were reinoculated intraperitoneally on the 27th day with 10^{-6} dilution of a suspension of *B. tularense* used in a later experiment (see Table V). The inoculum represented 10+ MLD. All mice died on the third or fourth day after reinoculation. Autopsies on three showed typical changes of tularemia. Spleen impression smears were positive for *B. tularense,* and the organism was recovered in pure culture from the spleens and heart bloods. There is thus no evidence that mice surviving inoculation with *B. tularense* for 27 days as a result of aureomycin treatment had any appreciable immunity on reinoculation.

A second experiment was undertaken to determine the effect of smaller subcutaneous doses of aureomycin (Table IV). The LD_{50} of the suspension was $10^{-8.5}$. Groups of mice were inoculated with 10^{-6} dilution, and treatment was begun two hours later. All of 10 untreated mice died on the third or fourth day. Animals treated with 0.5, 0.25, 0.125, and 0.065 mg. of aureomycin subcutaneously three times daily at about eight hour intervals for a total of 16 doses (*i.e.,* 1.5 to 0.19 mg./day, or approximately 75 to 9.4 mg./kg./day) remained well during five days therapy but all except three of 37 animals died after treatment was discontinued. That the deaths were due to tularemia was confirmed by positive autopsy findings and spleen impression smears. Mice receiving the largest dose of aureomycin (1.5 mg./day) survived longest. It did not seem feasible to

TABLE IV

EFFECT OF SUBCUTANEOUS AUREOMYCIN THERAPY BEGUN 2 HOURS AFTER INTRAPERITONEAL INOCULATION OF MICE WITH B. TULARENSE

DOSAGE S-C MG/D, 5 DAYS	NO. MICE INOC.	DEATHS BY DAYS AFTER INOCULATION			
		DAYS: 5	10	15	20
0	10	DEATHS: 9 1			
0.19	8		7 1		
0.375	10	R	4 4		
0.75	10		4 6		
1.5	9		1 2	1 1 1 1	1

LD_{50} OF SUSPENSION, $10^{-8.5}$
INOCULUM: 0.1 ML OF 10^{-6} I-P (10+ MLD)

use larger subcutaneous doses since edema, induration, and local necrosis were not infrequently noted following repeated injections of 0.5 mg. of aureomycin-HCl in 0.25 or 0.2 ml. of distilled water (2 to 2.5 mg./ml.). Local reactions were inconspicuous with concentrations of 1 mg./ml. or less.

The effect of duration and time of starting subcutaneous aureomycin treatment were next investigated (Table V). The LD_{50} of the suspension was $10^{-8.2}$. All of 20 control mice receiving the 10^{-6} dilution died on the third or fourth day. Groups of 10 mice which received the same inoculum were given 0.5 mg. of aureomycin subcutaneously at about eight hour intervals (1.5 mg./day or approximately 75 mg./kg./day) for 16 injections over a period of five days. One group received the first injection immediately before inoculation. A second group was started on therapy seven hours after inoculation. Two additional groups were given the same dosage of aureomycin started at the same times, but were continued on treatment for eight days or a total of 25 injections. All except one of the 40 treated mice remained well during therapy, but deaths began to occur on the fourth to sixth day after cessation of treatment and relatively few animals survived. Half of the group in which therapy was started just before inoculation and continued for eight days lived.

Two additional groups of 10 mice each, not shown in Table V, were included in this experiment and received the same inoculum at the same time. Treatment by subcutaneous injection was deferred until 28 hours after inoculation and continued for five and eight days respectively. These animals were likewise protected during therapy but deaths began to occur on the fifth day after cessation of treatment. All except one in the group treated five days and two in the group treated eight days succumbed.

The best result (50% survival) was thus obtained when subcutaneous therapy was begun immediately before inoculation and continued for the longest period, *i.e.*, eight days. Whether more complete protection could be obtained with larger doses administered for a longer period, or with repeated short courses of treatment, is not known.

TABLE V

EFFECT OF DURATION AND TIME OF STARTING AUREOMYCIN THERAPY (1.5 MG/D S-C) IN MICE INOCULATED INTRAPERITONEALLY WITH B. TULARENSE

TREATMENT		NO. MICE INOC.	DEATHS BY DAYS AFTER INOCULATION						
			DAYS: 5	10	15	20	25	30	
NONE		20	DEATHS: 14 6						
DURATION	ONSET								
5D	BEFORE INOC	10	1		5	1	1	1	1
5D	7 HRS LATER	10		1	8	1			
8D	BEFORE INOC	10	Rx		1 2	1			
8D	7 HRS LATER	10			5 2	2 1			

LD_{50} OF SUSPENSION, $10^{-8.2}$
INOCULUM 0.1 ML OF 10^{-6} 1-P (10+ MLD)

4. *Oral aureomycin therapy of experimental tularemia in mice*

Methods: Powdered drug from capsules was mixed with dry finely-ground Rockland mouse diet to yield concentrations of 0.5%, 0.25%, 0.125%, and 0.065%. Food was withheld for six hours and mice were then given the drug-containing diet *ad libitum*. By weighing the food taken it was found that 10 mice on the diet containing aureomycin 0.125%, inoculated intraperitoneally with *B. tularense* on the second day, consumed an average of 3.68 gm. per mouse per day over an eight day period. If the average consumption be assumed to approximate 3.5 gm. per mouse per day, the amount of aureomycin taken in these diets may be estimated roughly as follows:

% of aureomycin in diet	Estimated amount of aureomycin consumed per mouse per day
%	*mg.*
0.5	17.5
0.25	8.8
0.125	4.4
0.065	2.2

In therapeutic experiments the drug-containing diet was begun, after a six hour fast, on the day prior to intraperitoneal inoculation with *B. tularense*.

Procedure and Results: Groups of 10 mice were started on diets containing aureomycin 0.5%, 0.25%, 0.125%, and 0.065% while 20 control mice were given the basic diet without aureomycin. After 22 hours all mice were inoculated with a 10^{-6} dilution of a suspension of *B. tularense* (Table VI). The LD_{50} of the suspension was $10^{-8.2}$. The treated animals were fed the aureomycin-containing diets for 14 days. All of the control mice died on the third or fourth day after inoculation. None of the animals receiving drug in the diet died during 14 days of therapy, but deaths began to occur on the third to fifth days after the basic ration was resumed. Nevertheless, significant numbers remained alive; a survival rate of 60% was observed in the mice fed diet containing 0.5% of aureomycin, with somewhat fewer survivals in the other groups. These results compared favorably with those observed after subcutaneous treatment.

TABLE VI

EFFECT OF AUREOMYCIN IN DIET OF MICE INOCULATED INTRAPERITONEALLY WITH B. TULARENSE

% AUREOMYCIN IN DIET	NO MICE INOC	DEATHS BY DAYS AFTER INOCULATION (DAYS 5 10 15 20 25 30)
0	20	DEATHS 14 6
0.065	10	1 1 1 2 1
0.125	10	1 3 1 1 1
0.25	10	1 1 . 2 1 1
0.5	10	1 2 1

R_x IN DIET

LD_{50} OF SUSPENSION, $10^{-8.2}$
INOCULUM 0.1 ML OF 10^{-6} 1-P (10+ MLD)
DIET STARTED 22 HOURS BEFORE INOCULATION

In studies on aureomycin therapy of relapsing fever, Heilman (7) found that the oral dosage required was in the order of five times the subcutaneous dose. If the same ratio pertains in tularemia, the diet containing 0.25% aureomycin might be estimated to be about equivalent to subcutaneous dosage of 1.5 mg. daily. In our experiments the results obtained with this diet fed for 14 days approximated those with subcutaneous injections of 1.5 mg. daily for eight days beginning immediately before inoculation (cf. Tables V and VI).

A second feeding experiment was undertaken to investigate the effectiveness of aureomycin in suppressing larger inocula (Table VII). Six groups of 10 mice were started on a diet containing 0.065% aureomycin. After 22 hours these were inoculated with the 10^{-2} to 10^{-7} dilutions of a suspension of *B. tularense*. Control groups of six mice fed on basic ration without aureomycin were simultaneously inoculated with the 10^{-2} to 10^{-10} dilutions. The treated animals received the drug-containing diet until eight days after inoculation, when it was replaced by basic ration. The LD_{50} of the suspension was $10^{-8.6}$. All of the control mice inoculated with the 10^{-2} to 10^{-8} dilutions died on or before the fifth day. In the group of treated mice which received the 10^{-2} dilution, representing about 1,000,000 MLD, five deaths occurred during the eight day treatment period, but only one of 50 mice in the other groups died during therapy. Partial suppression of tularemia was thus demonstrated by aureomycin against 1,000,000 MLD, and almost complete suppression was observed with inocula ranging from 10 to 100,000 MLD. However, after the drug-containing diet was withdrawn deaths began to occur and most of the animals succumbed by the 13th day after inoculation. The greatest percentage of survivals was noted in the group given the smallest inoculum, *i.e.*, the 10^{-7} dilution, which contained about 10 MLD.

The size of inoculum appeared to be a decisive factor in determining ultimate survival of mice infected intraperitoneally with *B. tularense* and given aureomycin therapy, either orally or subcutaneously. With small inocula ranging from one to 10 MLD a very significant number of animals remained well. Although the drug exerted a striking suppressive effect against inocula up to 100,000 MLD, few mice which received inocula approaching or exceeding 100 MLD survived.

TABLE VII

SUPPRESSIVE EFFECT OF AUREOMYCIN 0.065% IN DIET OF MICE INFECTED WITH VARYING INOCULA OF B. TULARENSE

DILN INOC (0.1 ML I-P)	NO INOC	DIET	DEATHS BY DAYS AFTER INOCULATION (DAYS 5 10 15 20 25 30)	
10^{-2}	6	NORMAL RATION	DEATHS: 5 1	1,000,000 MLD
10^{-3}	6		2 4	100,000 MLD
10^{-4}	6		1 5	10,000 MLD
10^{-5}	6		4 2	1,000 MLD
10^{-6}	6		1 5	100 MLD
10^{-7}	6		6	10 MLD
10^{-8}	6		6	1 MLD
10^{-9}*	6		1 1	
10^{-2}	10	AUREO 0.065%**	1* 1 1+2 1 2 2	
10^{-3}	10		5 4 1	
10^{-4}	10		2 1 5 1 1 1	
10^{-5}	10		1 1 1 5 1 1	
10^{-6}	10		5 2 1	
10^{-7}	10		2 1 2 1	

R_x IN DIET

LD_{50} OF SUSPENSION, $10^{-8.75}$
* SIX UNTREATED MICE RECEIVING 10^{-10} SURVIVED.
** DIET STARTED 22 HOURS BEFORE INOCULATION

DISCUSSION

The first successful chemotherapy of experimental tularemia was reported by Heilman (8). He administered streptomycin subcutaneously to mice in divided doses totalling 1 mg. daily. Four injections of 0.15 mg. in saline and one of 0.4 mg. in beeswax and sesame oil were given in each 24 hours, and continued for 10 days beginning seven or eight hours after intraperitoneal injection of *B. tularense*. This regimen protected mice against inocula estimated at 10+ to 100+ MLD. When the total daily dose of streptomycin was reduced to 0.5 mg., only five of 12 mice were protected against 100+ MLD. The strain of *B. tularense* used was reported to be completely inhibited by 0.15 microgram of streptomycin per ml. *in vitro*. Because of differences in dosage form and schedule it is not possible to make a direct comparison between the results with aureomycin

reported herein and those obtained by Heilman using streptomycin.

Comparative studies on the activity of aureomycin, streptomycin, and chloromycetin in experimental tularemia have recently been reported by Woodward *et al.* (9). Using larger doses of aureomycin (3 mg./day intramuscularly for four days) they also occasionally obtained 100% survival of mice infected intraperitoneally with small inocula, but with larger inocula death regularly occurred after cessation of aureomycin treatment. They concluded, however, that aureomycin was more effective than streptomycin or chloromycetin in delaying death of mice infected with *B. tularense.*

Because experimental tularemia in the mouse is an overwhelming septicemic disease with 100% mortality, it is a severe test of a chemotherapeutic agent. It is difficult to apply the results obtained to the human disease, either in general terms or in the more delicate matter of dosage required. When significant activity against *B. tularense* has been established, both *in vitro* and in mice, carefully controlled clinical trial is the only means of evaluating the usefulness of a new antibiotic in human tularemia.

Woodward *et al.* (9) reported three tularemia patients in whom aureomycin was considered to be effective, and we have also treated three cases with apparently good results (1). Further cautious clinical trial of aureomycin in human tularemia is strongly indicated.

SUMMARY

Aureomycin exerted definite activity against a virulent strain of *B. tularense, in vitro* and *in vivo.* When the drug was incorporated into blood dextrose cystine agar, *B. tularense* was inhibited by 0.5 microgram per ml. at 24 hours and 2 micrograms per ml. at 48 hours. Its sensitivity was comparable to that of a strain of *Staph. aureus.* In concentrations of 8 micrograms or less per ml., aureomycin was bacteriostatic. However, when a suspension of *B. tularense* was incubated for four hours in the presence of 500 micrograms per ml., appreciable numbers of organisms were killed as demonstrated by culture and titration in mice.

Furthermore, aureomycin administered subcutaneously or orally demonstrated a striking suppressive effect in mice inoculated intraperitoneally with *B. tularense.* Almost all animals survived treatment periods ranging from five to 14 days, but most of them died after cessation of therapy. With inocula between 10 and 100 MLD, about 50% survival resulted from subcutaneous therapy with 1.5 mg./day (75 mg./kg./day) given in three divided doses starting immediately before inoculation and continued at about eight hour intervals for eight days. Treatment achieved 100% protection in small groups of mice inoculated with 1 to 10 MLD.

Administration of 0.065% aureomycin in the diet of mice infected intraperitoneally with *B. tularense* produced partial suppression of 1,000,000 MLD and almost complete suppression of 10 to 100,000 MLD during eight days therapy but most of the animals died after treatment was discontinued. Four of ten treated animals receiving only 10 MLD survived.

A careful trial of aureomycin in therapy of human tularemia is indicated.

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