TABLE V. Mortality Rates After Injection of Tri-Ethylene Melamine.*

Drug	Each dose, mg/kg	Proportion dying, 0-8th day	Mortality rate, 0-8th day	Proportion dying, 15-31st day	Mortality rate, 15-31st day	Column 6 / Column 4
Column 1	2	3	4	5	6	7†
Tri-ethylene	.25	1/4		0/3		
melamine	3	1/6		4/5		
in 2 doses	4	0/6		2/3		
	5	4/5		0/1		
		6/21	.29	6/12	.50	+1.72
Tri-ethylene	4	10/17		0/7		
melamine	5	12/17		0/5		
in 1 dose						
		32/34	.97	0/12	0	0

* Only data satisfying the following conditions used: Dose caused 20% or more deaths; dose did not cause more than 80% deaths during first 8 days.

† Column 7 represents: Death rate for later period—15-31st (Column 6) over death rate for earlier period—0-8th days (Column 4).

count and decrease in percentage of mononuclear cells. However, the return to a normal blood picture was no indication that the effects of the drug had been extinguished.

Received December 22, 1950. P.S.E.B.M., 1951, v76.

Aureomycin and Terramycin Treatment of *Pasteurella multocida* Infection and Neomycin's *in vitro* Effects on *P. multocida*.* (18534)

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Pasteurella multocida is primarily an animal pathogen. Recent evidence, however, indicates that it is encountered in infections of man more commonly than is generally realized(1,2). For this reason the determination of its susceptibility to various antibiotics is of interest. It was shown in a comparative study, using 8 strains, that *in vitro* aureomycin, chloromycetin, terramycin, polymyxin B and penicillin are highly effective, that streptomycin is somewhat less efficacious and bacitracin ineffective against this microorganism(3). Independently, Prier(4) reported that penicillin and streptomycin are somewhat inferior to aureomycin. Little(5) and Prier (4) found aureomycin to be effective in experimental infection of 1-day-old chicks and chick embryos. The experiments to be reported here were carried out to determine (1) the prophylactic and therapeutic efficacy of terramycin in experimental *P. multocida* infection of mice, (2) the *in vivo* effectiveness of aureomycin in an experimental animal other than previously reported, namely, the mouse and (3) the *in vitro* bacteriostatic potency of neomycin, which as yet has not been investigated.

Material and methods. Of the strains of *P. multocida* available at this laboratory and

* Aided in part by a grant of Lederle Laboratories Division, American Cyanamid Co.

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4. Prier, J. E., *Vet. Med.*, 1950, v45, 243.

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TABLE I. Prophylactic and Therapeutic Effects of Aureomycin and Terramycin in Experimental *Pasteurella multocida* Infection of Mice.

Drug	Dosage (mg)	No. of doses (q. 48 hr)	Proportion of animals dead on days after infection			
			3rd	6th	10th	15th
Aureomycin	2	1 P*	4/64†	7/64	12/64	13/64
	2	4 P	0/72	0/72	1/72	2/72
	2	1 T‡	0/61	2/61	2/61	3/61
	2	4 T	1/62	8/62	11/62	19/62
	2	2 P	2/44	2/44	3/44	4/44
	2	2 T	7/52	13/52	16/52	17/52
	1	1 P	3/58	25/58	32/58	33/58
	1	1 T	0/39	2/39	16/39	31/39
	.5	1 P	5/34	30/34	31/34	31/34
	.5	1 T	5/34	23/34	26/34	26/34
	.2	2 P	32/60	36/60	37/60	37/60
Terramycin	2	1 P	7/108	25/108	51/108	54/108
	2	4 P	13/119	22/119	34/119	45/119
	2	1 T	0/150	35/150	54/150	82/150
	2	4 T	12/129	49/129	68/129	73/129
	1	1 P	45/122	93/122	110/122	120/122
	1	4 P	20/51	21/51	35/51	41/51
	1	1 T	15/102	73/102	75/102	75/102
	1	4 T	3/57	5/57	9/57	10/57
	.5	1 P	34/54	46/54	47/54	50/54
	.5	4 P	19/22	19/22	19/22	19/22
	.5	1 T	33/54	45/54	47/54	47/54
	.5	4 T	16/22	17/22	19/22	19/22
	.2	1 P	37/40	38/40	38/40	38/40
	.2	4 P	35/36	35/36	35/36	35/36
	.2	1 T	20/20	20/20	20/20	20/20
	.2	4 T	43/56	46/56	46/56	46/56
Control	—	—	212/212	212/212	212/212	212/212

*P = First dose of antibiotic at time of infection.
† Numerator = No. of dead animals. Denominator = Total No. of animals.
‡T = First dose of antibiotic 4 hr after infection.

tested for their sensitivity to 7 antibiotics several proved to be of low virulence in mice, while others, even in small numbers, produced a rapidly fatal infection. Strain No. 200, kindly supplied by Dr. Brunner of Cornell University and identified as *P. avicida,* was used throughout this study. It was maintained on blood agar by means of weekly transfers. For the experimental infection 18 hour-old infusion broth cultures were used in suitable dilutions; the dilutions were prepared in infusion broth and immediately injected intraperitoneally (vol. 0.5 ml) into white mice, weighing between 15 and 18 g. Aureomycin hydrochloride (intravenous) was made available through the courtesy of Dr. B. W. Carey of Lederle Laboratories, terramycin hydrochloride was kindly supplied by Dr. Elliott R. Weyer of Chas. Pfizer and Co. and neomycin sulfate by Dr. H. F. Colfer of Merck and Co. The drugs were dissolved in physiological saline solution and used within a few hours; they were administered subcutaneously (vol. 0.2 ml) in order to preclude direct contact between the antibiotic and the injected organisms. The determination of the *in vitro* bacteriostatic potency of neomycin on 8 strains of *P. multocida* was carried out according to the method previously described (3).

Results. Preliminary to the study on the *in vivo* efficacy of terramycin and aureomycin in mice infected experimentally with *P. multocida,* the MLD_{100} and MLD_{50} were determined. On the basis of experiments on 312 mice it was found that 0.5 ml of an 18-hour broth culture diluted up to 1×10^8 caused death of all animals within 3 days and that 0.5 ml of such a broth culture diluted 1 to 5×10^{10} caused death in approximately 50% of the animals. The majority of experiments with antibiotics were carried out with a broth

culture diluted 100,000 fold; the infected inoculum, therefore, contained approximately 1,000 MLD_{100} or 100,000 to 500,000 MLD_{50}. The results of aureomycin and terramycin in experimental *P. multocida* infection of white mice are summarized in Table I. A perusal of this table indicates the following: (1) All untreated animals died within 3 days; the majority succumbed between 24 and 48 hours following the infection. (2) Both antibiotics are effective in delaying death and lowering the death rate during the 15-day period of observation. (3) Aureomycin is distinctly superior to terramycin, inasmuch as only 58 (ca 16%) out of 355 mice treated with aureomycin in a dosage of 2 mg (133 mg/kg) (given once, twice and 4 times) succumbed, whereas 254 (ca 50%) out of 506 animals died when treated with terramycin. With a single 2 mg dose, the respective figures are 16 (ca 13%) out of 125 mice treated with aureomycin and 136 (ca 53%) out of 258 animals treated with terramycin.† (4) That a single 2 mg dose, prophylactically or therapeutically, is superior to single 1 mg and 0.5 mg doses with both antibiotics is evident from the fact that the fatality rates for aureomycin are respectively 13%, 66%, and 84% and those for terramycin 53%, 87%, and 90%.† These figures also reveal the greater efficacy of aureomycin in the 2 mg and 1 mg doses.† (5) Delay of treatment for 4 hours after the infection does not materially affect the outcome. (6) Treatment for 6 days (4 doses 48 hours apart) does not substantially increase the survival rate on the 15th day when the latter is compared with that of 6th and 10th day of the groups of animals which received only a single dose of the antibiotic on the day of the infection. This data together with that obtained by Little(5) and Prier(4) suggests that aureomycin may be useful in the treatment of human *P. multocida* infections, which appear to be more common than is presently realized. The therapeutic efficacy of this antibiotic in a localized *P. multocida* infection of man is being reported elsewhere(6).

Since it was observed recently in a comparative study on the bacteriostatic activity of 7 antibiotics upon 8 representative strains of *P. multocida* that streptomycin was somewhat less effective than aureomycin, chloromycetin, terramycin, polymyxin B and penicillin, it was deemed of interest to determine the growth inhibitory activity of neomycin upon these strains. Using the technic previously described(3), it was found that the bacteriostatic potency of neomycin was of the same order of magnitude as that of streptomycin, inasmuch as the minimal inhibitory concentration of neomycin ranged from 6 to 25 μg per ml and that of streptomycin was 10 μg/ml with 7 out of 8 strains studied. The eighth strain was highly resistant to streptomycin, growing profusely in the presence of 10,000-50,000 μg per ml; the patient from whom this strain was isolated had not previously received streptomycin. In contrast, this strain was susceptible to neomycin; 0.3 μg/ml prevented its growth.

Summary. (1) Both aureomycin and terramycin administered subcutaneously, either prophylactically or therapeutically (4 hours after infection) to white mice infected intraperitoneally with 1,000 MLD_{100} of *P. multocida* prolonged survival and lowered the fatility rate. Aureomycin was distinctly superior to terramycin; a single dose of 2 mg (133 mg/kg) of aureomycin resulted in a survival rate of approximately 87%, whereas that of the mice treated with terramycin was approximately 47%. Smaller doses (1 mg and less) of both antibiotics were less effective. Treatment over a period of 6 days did not increase substantially the survival rate as compared to a single dose treatment on day of infection. (2) *In vitro* neomycin was of the same bacteriostatic potency as streptomycin against 7 strains of *P. multocida* and was markedly superior against the eighth, streptomycin-resistant, strain.

† The above corresponding figures were analyzed statistically, and found to be significantly different, by John Neter of Syracuse University, to whom the authors are indebted.

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Received January 9, 1951. P.S.E.B.M., 1951, v76.