ANTIBACTERIAL ACTIVITY OF EFROTOMYCIN

B. M. FROST, M. E. VALIANT, B. WEISSBERGER and E. L. DULANEY

Merck Institute for Therapeutic Research Rahway,
New Jersey, 07065 U.S.A.

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Efrotomycin is a narrow spectrum antibiotic. Among the genera tested for susceptibility *in vitro* it is most active against isolates of *Moraxella, Pasteurella, Yersinia, Haemophilus, Streptococcus* and *Corynebacterium*. The drug is as active by oral administration as by the subcutaneous route. Blood levels rise rapidly to high concentrations, after oral dosing, and are prolonged. Two peaks occur which may indicate biliary excretion and reabsorption. Urinary excretion is minimal. The high blood concentrations explain, in part, the *in vivo* activity against pathogens such as *Bordetella bronchiseptica* which are relatively insensitive *in vitro*. Oral activity of efrotomycin is an advantage over the related antibiotics, X-5108 and mocimycin.

Three related antibiotics X-5108[1], mocimycin[2,3], and kirromycin[4], were described in 1972~1973. Mocimycin and kirromycin are identical and antibiotic X-5108 is their N-methylated form[5]. Efrotomycin[6] belongs to this family of antibiotics. It is a disaccharide derivative of antibiotic X-5108 (DEWEY, R. S. and G. ALBERS-SCHONBERG, personal communication). The absolute structure of efrotomycin will be presented in a forthcoming paper. In the present communication, we report some *in vitro* and *in vivo* activities of efrotomycin (greater than 90% pure), and some comparisons with antibiotic X-5108 and mocimycin are made.

In Vitro Activity

Antibacterial Spectrum

Initial tests showed efrotomycin to have a limited but interesting spectrum in that it was active *in vitro* and *in vivo* against some important animal pathogens. The *in vitro* testing was then extended to a series of human and animal pathogens. In these studies, the test bacteria were grown for 16 hours in brain-heart broth (including 5% horse serum when necessary), diluted to 10^{-3} in fresh medium and spot-inoculated onto the surface of 100 mm plates containing 10 ml of brain-heart agar with various concentrations of the antibiotic. The number of viable cells inoculated onto the plates varied from 3×10^2 to 7×10^3 per spot, depending on the test organism. The plates were scored visually after 24 and 48 hours.

The results in Table 1 show that efrotomycin is not very active *in vitro*. Strains of *Moraxella, Pasteurella, Yersinia, Haemophilus, Streptococcus* and *Corynebacterium* are the most sensitive. The limited data with X-5108 and mocimycin show these two antibiotics to have a spectrum similar to efrotomycin but to be more active than efrotomycin *in vitro*.

Bactericidal Activity

The question as to whether efrotomycin was bactericidal or bacteriostatic was answered by the following experiments. Test bacteria were grown for 16 hours in brain-heart broth, diluted in fresh broth and added to tubes of brain-heart broth containing the antibiotic. The final concentration of cells varied from 1.8×10^6 to 1.9×10^7 per ml, depending on the test organism. The tubes were examined visually after 24 hours incubation at 37°C. The minimal inhibitory concentration(MIC) was taken as the lowest

Table 1. Antibacterial spectrum of efrotomycin with some comparisons with X-5108 and mocimycin

Test organism	Code No.	Animal source	MIC (μg/ml)							
			Efrotomycin				X-5108		Mocimycin	
			No serum		Plus serum		Plus serum		Plus serum	
			24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Aerobacter sp.	3309	human	>400	>400						
Aerobacter sp.	3352	human	>400	>400						
Aerobacter sp.	3253	human	>400	>400						
Bordetella bronchiseptica	F1728	porcine	150	150	150	150	25	50	25	100
Bordetella bronchiseptica	25	porcine	100	150						
Bordetella bronchiseptica	26	porcine	100	100						
Bordetella bronchiseptica	29	porcine	250	300						
Bordetella bronchiseptica	39	porcine	100	100						
Bordetella bronchiseptica	38	porcine	100	100						
Bordetella bronchiseptica	48	porcine	200	200						
Bordetella bronchiseptica	65	porcine	100	100						
Bordetella bronchiseptica	74	porcine	100	100						
Bordetella bronchiseptica	76	porcine	250	250						
Bordetella bronchiseptica	77	porcine	200	200						
Bordetella bronchiseptica	81	porcine	100	150						
Corynebacterium equi	223	equine			25	25				
Corynebacterium ulcerans	225	?			25	25				
Corynebacterium hofmannii	226-1	?			50	50				
Corynebacterium hofmannii	226-2	?			50	50				
Corynebacterium renale	227-1	?			50	50				
Corynebacterium renale	227-2	?			50	50				
Corynebacterium renale	227-3	?			50	50				
" *diphtheriae gravis*	3176	human	25	25						
" *pseudotuberculosis*	3165	equine			50	100	3.12	25	3.12	25
" *pyogenes*	516	?			N.G.	6.25	N.G.	3.12	N.G.	3.12
Escherichia coli	2908	porcine	150	200						
Escherichia coli	3385	porcine	100	150						
Escherichia coli	3392	porcine	400	400						
Escherichia coli	2017	human	400	>400						
Escherichia coli	3386	bovine	350	350			250	>400	150	200
Erysipelothrix sp.	166	?	250	400						
Erysipelothrix rhuziopathiae	87193	porcine	250	400			350	>400	>400	>400
Erysipelothrix rhuziopathiae	84	avian	250	>400						
Erysipelothrix rhuziopathiae	100	avian	250	>400						
Hemophilus influenzae	2261	human			12.5	12.5	0.39	0.78	0.39	1.56
Klebsiella pneumoniae	3083	human	>400	>400			350	350	>400	>400
Klebsiella pneumoniae	3068	human	150	150						
Moraxella bovis	2284	bovine			0.39	0.39	<0.097	<0.097	<0.39	<0.39
Moraxella bovis	418	bovine			0.19	0.19	<0.048	<0.048	<0.39	<0.39
Moraxella bovis	419	bovine			0.39	0.39	<0.048	<0.048	<0.39	<0.39
Moraxella bovis	420	bovine			0.19	0.19	<0.048	<0.048	<0.39	<0.39

Paracolobactrum sp.	3335	human	>400	>400			350	>400	>400	>400
Paracolobactrum sp.	3341	human	>400	>400						
Pasteurella haemolytica	12	bovine	6.25	6.25						
Pasteurella haemolytica	67	bovine	6.25	6.25						
Pasteurella multocida	X–73	avian	6.25	6.25						
Pasteurella multocida	1590	equine	3.122	3.12						
Pasteurella multocida	443–68	avian	3.12	3.12						
Pasteurella multocida	8579	avian	3.12	3.12						
Pasteurella multocida	86	avian			6.25	6.25	0.78	3.12	3.12	3.12
Pasteurella multocida	8608	avian			6.25	6.25				
Pasteurella multocida	89	avian			6.25	6.25				
Pasteurella multocida	9481	avian			6.25	6.25				
Pasteurella multocida	2909	avian			6.25	6.25				
Pasteurella multocida	2871	bovine			6.25	6.25				
Pasteurella multocida	2869	avian			25	25				
Proteus inconstans	2741	human			>400	>400	150	250	>400	>400
Proteus mirabilis	3201	human	350	350						
Proteus mirabilis	2919	human	200	200						
Proteus mirabilis	2915	human	>400	>400						
Proteus mirabilis	2918	human	400	>400						
Proteus mirabilis	3011	human	250	250						
Proteus mirabilis	3327	human	>400	>400						
Proteus vulgaris	1810	human	300	350						
Proteus vulgaris	3314	human	350	350						
Pseudomonas aeruginosa	3210	human	>400	>400			300	>400	>400	>400
Pseudomonas aeruginosa	3301	human	>400	>400						
Pseudomonas aeruginosa	3250	human	>400	>400						
Salmonella schottmuelleri	3010	human	300	300						
" *cholerasuis kunzendorf*		porcine	350	350						
Salmonella enteritis	3421	?	>400	>400						
Salmonella decatur	60AF	porcine	400	>400						
Salmonella sp.		?	>400	>400						
Salmonella typhimurium	3404	?	>400	>400						
Salmonella typhimurium	3420	?			>400	>400	200	350	200	>400
Serratia marcescens	3374	human	350	350						
Serratia marcescens	1543	human	300	300			>400	>400	>400	>400
Serratia marcescens	1544	human	250	250						
Serratia marcescens	1545	human	250	300						
Serratia marcescens	1546	human	250	250						
Serratia marcescens	1547	human	250	250						
Shigella sp.	3303	human	>400	>400			100	100	150	200
Shigella sp.	3304	human	>400	>400						
Shigella sp.	3371	human	>400	>400						
Shigella sp.	3297	human	>400	>400						
Staphyloccus aureus	2949	human	>400	>400	>400	>400	200	350	300	400
Staphyloccus aureus	3089	human	>400	>400						
Staphyloccus aureus	3000	human	>400	>400						
Staphyloccus aureus	53	bovine	>400	>400						
Staphyloccus aureus	2957	human	>400	>400						

(to be continued)

Table 1. (Contd.)

Test organism	Code No.	Animal source	MIC (μg/ml)							
			Efrotomycin				X-5108		Mocimycin	
			No serum		Plus serum		Plus serum		Plus serum	
			24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Streptococcus pneumoniae	I-37	human			50	50	0.78	3.12	0.78	3.12
Streptococcus pneumoniae	II-3372	human			12.5	25				
Streptococcus pneumoniae	III-3373	human			12.5	25				
Streptococcus pyogenes	C-203	human			50	50	0.39	1.56	<0.4	>3.12
Streptococcus pyogenes	3332	human			1.56	6.25				
Streptococcus pyogenes	1685	human			50	50				
Streptococcus pyogenes		human			1.56	1.56				
Streptococcus Group D	198	bovine	>400	>400			200	300	>400	>400
Streptococcus Group D	199	bovine	>400	>400						
Streptococcus Group D	200	bovine	>400	>400						
Streptococcus Group D	201	bovine	>400	>400						
Streptococcus Group D	203	bovine	>400	>400						
Yersinia enterocolitica	WA	?			50	100	50	100	25	150
Yersinia pseudotuberculosis	271	avian	25	25						
Yersinia pseudotuberculosis	272	avian	25	25						
Yersinia pseudotuberculosis	273	rabbit	25	25						
Yersinia pseudotuberculosis	274	avian	25	25						
Yersinia pseudotuberculosis	275	avian	12.5	25			12.5	25	12.5	25
Yersinia pseudotuberculosis	276	avian	12.5	12.5						
Yersinia pseudotuberculosis	277	?	12.5	12.5						
Yersinia pseudotuberculosis	278	?	25	25						
Yersinia pseudotuberculosis	279	?	25	25						
Yersinia pseudotuberculosis	280	?	25	25						
Yersinia pseudotuberculosis	281	?	12.5	12.5						

* Received from clinic as *aerobacter*. Based on BERGEY's Manual of Determinative Bacteriology, 8th edition (1974), these isolates will have to be reclassified.
N.G.=No Growth.

Table 2. Bactericidal action of efrotomycin

Test organism	Code No.	MIC μg/ml	MLC μg/ml	Ratio MLC: MIC
Pasteurella multocida	86	6.2	6.2	1
Pasteurella multocida	89	12.5	50	4
Pasteurella multocida	9481	25	25	1
Pasteurella multocida	2871	6.2	25	4
Pasteurella multocida	8579	12.5	25	2
Bordetella bronchiseptica	F1728	200	400	2

MIC=Minimal inhibitory concentration; MLC=Minimal lethal concentration

level which prevented visible growth. One-tenth ml portions were removed from the tubes showing no growth and plated in 9.9 ml of brain-heart agar. After 72 hour incubation at 37°C, the colonies were counted. The minimum lethal concentration (MLC) was taken as the lowest efrotomycin concentration which killed 99.9% of the cells[7]. Data are summarized in Table 2.

It is clear that efrotomycin is bactericidal with the -static and -cidal concentrations being much the same. Studies on the kinetics of the bactericidal action of efrotomycin have yielded some interesting results. In these experiments, the test bacteria for preparing the inoculum were grown in shaken culture

Fig. 1. Effect of efrotomycin on the growth of a larger inoculum of *Moraxella bovis* 418

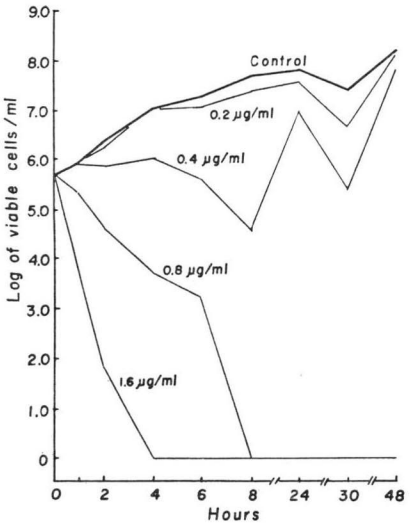


Fig. 2. Effect of efrotomycin on the growth of a small inoculum of *Moraxella bovis* 418

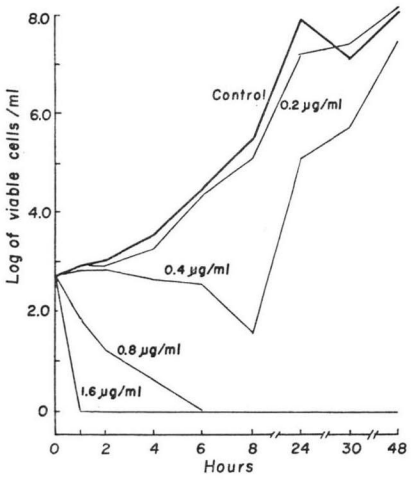


Fig. 3. Effect of efrotomycin on the growth of a larger inoculum of *Pasteurella multocida* 86

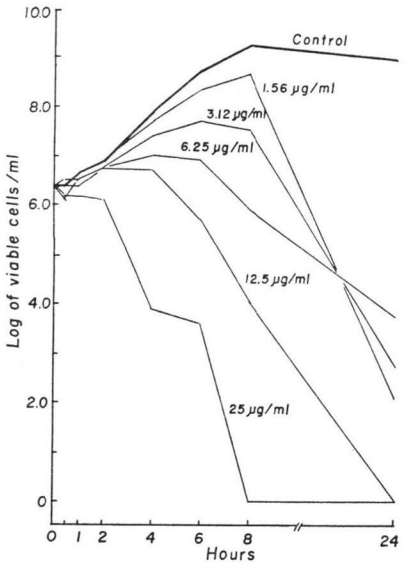


Fig. 4. Effect of efrotomycin on the growth of a small inoculum of *Pasteurella multocida* 86

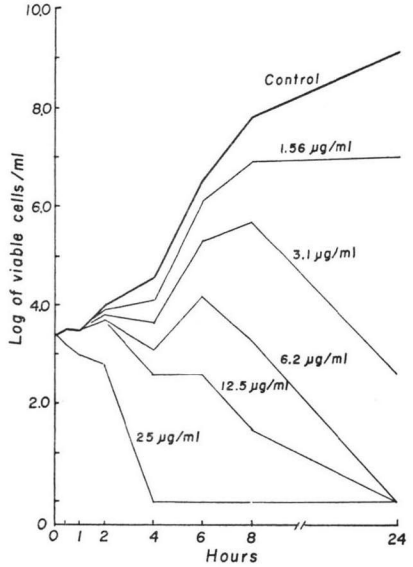


Table 3. *In vivo* antibacterial activity of efrotomycin

Infecting culture		Source	MIC	Approximate infecting dose		ED_{50} - mg/dose - 2 doses						
						Efrotomycin		Na Pen G		Sulfamethazine	Chlortetracycline	Chloramphenicol
			μg/ml	LD_{50}s	c.f.u.	s.c.	p.o.	s.c.	p.o.	p.o.	p.o.	p.o.
Pasteurella multocida	2871	bovine	6.2	7	6	0.52	0.71	0.03	0.15			
	8579	avian	12.5	14	9.8×10^{1}	0.77	1.77	0.04	0.09			
	8608	avian	6.2	9	5	1.36	1.65	0.05	0.16			
	86	avian	6.2	73	3.2×10^{2}	0.41	0.45	0.06	0.29			
	89	avian	12.5	9	1.9×10^{1}	0.76	0.82	0.04	0.14			
Bordetella bronchiseptica	F1728	porcine	150	7	1.2×10^{7}	0.71	1.4			0.015	(0.36 in a different test)	
	25	porcine	150	51	2.2×10^{8}		2.73			>10	0.31	4.01
	26	porcine	100	31	9.2×10^{7}		2.73			>20	0.47	ca 2.0
	48	porcine	200	31	4.7×10^{6}		4.16			(>5)	0.38	4.01
	65	porcine	100	7	4.9×10^{7}		3.27				0.19	0.92
	74	porcine	100	51	6.0×10^{6}		2.75			0.19	ca 0.46	3.16
	B	porcine		14	1.7×10^{7}		2.06			<0.04	0.56	1.03
Moraxella bovis	418	bovine	0.19	7	1.3×10^{7}		0.58	0.004			0.06	
	419	bovine	0.39	3	8.7×10^{7}		0.68	0.10			0.10	
	420	bovine	0.19	7	2.5×10^{8}		0.41	0.094			0.08	
	526	bovine	?	11	3.8×10^{7}		0.91	0.02			0.13	
	2884	bovine	0.39	3	2.5×10^{8}		0.57	0.06			<0.08	
Streptococcus pyogenes	C203	human	50	164	5.12×10^{2}	0.25	0.26	0.002	0.008			
Streptococcus Group D	203	human	>400	3	4.2×10^{8}		>6.0	0.08			>6.0	0.24
Streptococcus pneumoniae	I-37	human	100	51	6.5×10^{1}	>8.0	>8.0	0.004	0.034			
Staphylococcus aureus	Smith	human	>400	7	1.6×10^{2}		>6.0	0.02				
Escherichia coli	2017	human	400	7	8.8×10^{7}		>6.0				1.69	0.62
Salmonella schottmuelleri	3010	human	300	11	2.2×10^{7}		>6.0			3.57	0.76	0.18

* c.f.u.=colony forming units.

in brain-heart broth for 16 hours at 37°C, the same conditions used for the inoculum in the antibacterial spectrum and bactericidal studies. Two test organisms, *Moraxella bovis* 418 and *Pasteurella multocida* 86, were used at two dilution levels in brain-heart broth. Cultures with and without antibiotics were incubated and sampled at intervals, and plate counts were made for survival determinations.

Effective killing of *M. bovis* 418 depends on the inoculum concentration and requires 6~8 hours at a concentration of 0.8 μg of efrotomycin/ml. At an efrotomycin concentration of 0.4 μg/ml the bacterial counts are fairly level for 6 hours before dropping for a short period (~2 hours) prior to recovery and multiplication (Figs. 1 and 2). The relatively slow rate of kill by efrotomycin is also clear from the experiments with *P. multocida* 86 (Figs. 3 and 4). Most interesting is the observation that the bacteria can grow for several hours in efrotomycin concentrations that eventually kill most of the population. Concentrations of 1.56, 3.12 and 6.25 μg/ml in Fig. 3 and concentrations of 3.12 and 6.25 μg/ml in Fig. 4 show this phenomenon.

The only published data on the mode of action of this class of compounds are those of WOLF *et al.*[8] on kirromycin. This antibiotic is a potent inhibitor of bacterial protein synthesis by interfering with peptide transfer reactions associated with the elongation factor Tu. If this is the mechanism of action of efrotomycin as well, the effect is not immediately lethal except at relatively high concentrations of the drug.

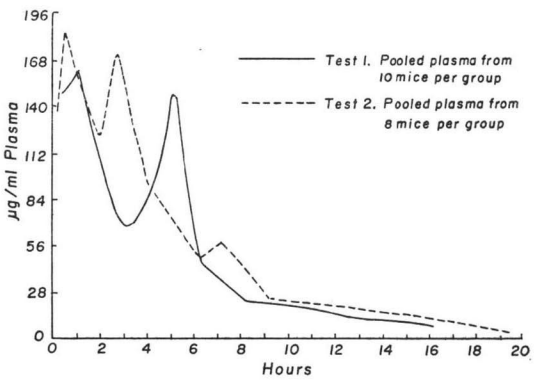
In Vivo Activity

Efficacy against Mouse Infections

Mice were injected intraperitoneally with cultures grown for 16 hours at 37°C in brain-heart broth. Efrotomycin, antibiotic X-5108 and mocimycin were dissolved in ethanol and diluted with 1% Tween 80 in water (v/v). The other drugs were administered as sonicates in 1% Tween, except for penicillin which was an aqueous solution. Five-tenths ml of drug was given by gavage or subcutaneously at the time of infection and 6 hours post infection. Groups of five CFl female mice were used for each level and were observed daily for 7~14 days after infection. The ED_{50} and LD_{50} were calculated by the method of KNUDSEN and CURTIS[9] as mg/dose/mouse. The results are summarized in Table 3.

Clearly, efrotomycin is rapidly absorbed after oral administration since it is as active by the oral route as by the subcutaneous route. The *in vivo* efficacy against *Bordetella bronchiseptica* is interesting in relation to the relative *in vitro* insensitivity of *B. bronchiseptica* isolates to the antibiotic. Moreover, efrotomycin is active against sulfamethazine-resistant *B. bronchiseptica* strains and is equal to chloramphenicol but less active than chlortetracycline. It is less active than penicillin G against strains of *P. multocida* and *M. bovis*.

Fig. 5. Plasma levels in mice following single oral administration of 200 mg efrotomycin per kg.



Supplies of antibiotic X-5108 and mocimycin were adequate for comparison with efrotomycin in only one infection. Both antibiotic X-5108 and mocimycin had lower MIC's than efrotomycin against *B. bronchiseptica* F1728.

However, two 6-mg doses of antibiotic X-5108 were not effective either by gavage or subcutaneously against an infection with *B. bronchiseptica* F1728. This dose, the highest tested, did give a statistically significant prolongation of mean survival time by both routes but lower doses had no effect. Mocimycin was completely ineffective by either route at two doses of 6 mg/mouse. The ED_{50} of efrotomycin in this test was 1.81 mg per dose by the oral route and 1.24 subcutaneously. Efrotomycin is well tolerated following either oral or subcutaneous administration. The oral LD_{50} is greater than 4 g/kg and the subcutaneous LD_{50} is greater than 2 g/kg.

Plasma Concentrations

The oral efficacy of efrotomycin against *B. bronchiseptica*, which is relatively insensitive *in vitro*, indicates that the antibiotic may be absorbed rapidly and reach and maintain high blood concentrations. This possibility was tested experimentally.

Randomized groups of CFl female mice were dosed by gavage with 4 mg of the sodium salt of efrotomycin per 20 g mouse. Blood was taken from the hearts at intervals using heparinized syringes and the plasma from each group was pooled and frozen until assayed. A microbiological cylinder agar plate diffusion assay was developed using *M. bovis* 418 as the test organism. Known concentrations of efrotomycin were prepared in normal mouse plasma and test samples were assayed against this standard. The data are presented in Fig. 5. The rapid absorption and prolonged high plasma levels of efrotomycin are evident. The two peaks of activity are most interesting. They have not been explained experimentally. However, biliary excretion of the drug followed by reabsorption is one possibility.

Serum Binding

Two ml volumes of 200 μg efrotomycin per ml of horse serum or saline were dialyzed in rotating chambers against 2 ml volumes of saline at 5°C for 48 hours. The test was performed in quadruplicate. Dialysates and dialysants were assayed for antibiotic content using appropriate standards and the *M. bovis* assay. Efrotomycin was 30% bound by horse serum under the conditions described.

Urinary Excretion

One reason for the prolonged blood levels of efrotomycin is the poor urinary excretion of the drug. This was shown experimentally as follows: Five Marland Farms female rats, varying in weight from 190 to 210 g each was given efrotomycin by gavage at a dose of 400 mg/kg body weight. A control group of rats received an equal volume of diluent and was held under the same test conditions. Food and water were available during the test except that water was withheld for the first 6 hours after dosing. Urine was collected from 0~6 hours and from 6~24 hours. Samples from five rats were pooled and frozen until assayed. Standards were prepared in normal urine. Only about 2% of the dose was recovered during the 24-hour test period with approximately 4/5 of this during the last 18 hours. The volume of urine was considerably less from the efrotomycin dosed rats than from the control group, particularly during the first 6 hours. Other studies have shown that continued dosing with high oral levels of efrotomycin have not caused the test rats to show urinary retention (H. M. PECK, personal communication).

Discussion

Efrotomycin is a narrow spectrum antibiotic with poor *in vitro* activity. However, the drug shows a number of favorable characteristics. It is quite active against several important animal pathogens. Indeed, its *in vivo* activity is greater than one would predict from *in vitro* potency. It is rapidly absorbed after oral administration and produces high prolonged blood levels; however, this is probably not the

only reason for the unexpected *in vivo* potency. It is not cross-resistant with other drugs used as feed additives or therapeutically in veterinary medicine. It offers promise as a growth permittant in the presence of disease complexes. Data on animal trials will be forthcoming from other investigators.

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