ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1978, p. 598-604
0066-4804/78/0013-0598$02.00/0

Vol. 13, No. 4
Printed in U.S.A.

Cetocycline, Tetracycline Analog: In Vitro Studies of Antimicrobial Activity, Serum Binding, Lipid Solubility, and Uptake by Bacteria

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Received for publication 7 December 1977

Cetocycline (formerly chelocardin or cetotetrine) is structurally related to the tetracyclines. It was found to be more active than tetracycline against many clinical isolates of aerobic gram-negative bacilli, but is less active against staphylococci, and has no activity against *Pseudomonas*. It is bactericidal against susceptible enteric gram-negative bacteria at concentrations two to four times higher than the minimal inhibiting concentrations. The drug is highly lipid soluble; more than 80% is bound to serum, and it is more avidly taken up by susceptible bacteria than tetracycline. A direct correlation between drug uptake and susceptibility of bacteria was not noted, except with a strain of *Proteus vulgaris*.

Cetocycline, formerly known as chelocardin or cetotetrine, is an antibiotic isolated from *Nocardia sulphurea*. It is structurally related to the tetracyclines, but contains a 9-methyl group, an aromatic C ring, an unsubstituted 4-ammonia group, and a methyl group replacing the 2-ammonia group (13). Its mode of action appears to be identical to that of tetracyclines, interfering with binding of amino acyl-ribonucleic acid to the 30S ribosomal units (12). Cetocycline is reported to be more active than tetracycline against various gram-negative aerobic bacteria, particularly *Proteus* species, but is not active against *Pseudomonas* and less active than other tetracyclines against gram-positive cocci (14).

This report presents comparative in vitro studies of the activity of cetocycline and tetracycline against a variety of aerobic bacteria isolated from clinical sources. Data is also presented on the effect of: (i) human serum on antimicrobial activity, (ii) binding by human serum, (iii) lipid solubility, and (iv) the relative uptake of the two drugs by susceptible and resistant organisms.

MATERIALS AND METHODS

Studies of antibacterial activity. Cetocycline was supplied by R. L. Girolami (Abbott Laboratories), and tetracycline was supplied from American Cyanamid Co., Lederle Laboratories Div. Stock solutions of 1 mg/ml were prepared in distilled water and stored at −20°C until used. Tube dilution tests were conducted in Trypticase soy broth (Difco) by adding 0.5 ml of broth containing a 1×10^{-4} dilution of an overnight culture to 0.5 ml of broth in which the drugs were serially diluted. This produced an inoculum of 1×10^5 to 5×10^5 colony forming units per ml. End points of minimal inhibitory concentrations (MICs) were read after 24 h of incubation at 37°C as the final dilution of the antibiotic preventing visible growth. Minimal bactericidal concentration (MBC) end points were obtained by pour plate cultures of all clear tubes using dilutions of 10^{-1}, 10^{-3}, and 10^{-5}. The MBC was designated as the highest dilution that reduced the inoculum greater than 10^3 times after overnight incubation.

A total of 300 isolates of aerobic enteric gram-negative bacteria, staphylococci, and non-group A streptococci were obtained from patient material isolated in the clinical microbiology laboratory at the William S. Middleton Veterans Administration Hospital. They were identified by standard bacteriological methods and subcultured on agar culture plates. Single colonies were picked, grown in Trypticase soy broth, and stored at −4°C. Fresh media were inoculated the evening before each test. On each test day, control cultures of *Staphylococcus aureus* ATCC 25923 and *Eschericia coli* ATCC 25922 were tested in an identical manner. Control organisms were also used to test the effect of pooled 50% fresh frozen human serum on the activity of cetocycline and tetracycline. In these experiments, both the inoculum and the drugs were diluted in this serum Tripticase soy broth medium.

Radiolabeled drugs. [^{3}H]cetocycline, specific activity 118.14 μCi/mg, was supplied by R. L. Fredrickson, Abbott Laboratories. [^{3}H]tetracycline as [7-^{3}H]-tetracycline hydrochloride, specific activity 2.5 μCi/mg and radiochemical purity 97%, was purchased from New England Nuclear Corp. The drugs were freshly

dissolved each day in phosphate buffer (pH 7.5) as 300-μg/ml stock solutions and then diluted further in test media. Radioactivity was measured by a Tri-Carb liquid scintillation counter (Packard Instrument Co.). Quenching was corrected by use of appropriate standards. All counts were corrected to disintegrations per minute by using an internal standard.

Serum binding studies. Serum binding studies were conducted by equilibrium dialysis, ultrafiltration, and ultracentrifugation. For equilibrium dialysis, 3 ml of pooled, fresh frozen human serum in cellophane bags was dialyzed against 3 ml of Krebs-Ringer phosphate buffer (pH 7.4) without added calcium, containing 0.2 μCi of radiolabeled drug and the desired amount of cold drug. Binding was calculated as follows: percent drug bound = 100 − free drug {[free drug (buffer)/total drug (bag)] × 100}; percent drug recovered = 100 × (counts recovered in buffer and bag/counts added to buffer).

For ultrafiltration, conical membranes (Centerflo, Amicon Corp., type CF-50) were soaked in Krebs-Ringer phosphate buffer. Excess buffer was removed by centrifugation (1,200 × *g* for 15 min), and the collecting tubes were wiped dry. Amounts of unlabeled and tritiated drug were then mixed with 9 ml of serum to achieve a 10-μg/ml concentration. At this point, 0.2 ml was removed to calculate total initial drug present. A 3-ml portion was placed in the filtration cone, and the rest was saved for ultracentrifugation. After incubation for 15 min at 23°C in the filtering cones, filtration was accomplished by centrifugation at 400 × *g* for 8 min. A 0.1-ml portion of ultrafiltrate was added to scintillation fluid to measure unbound drug. Any remaining filtrate was carefully returned to the filtration cone. After mixing, a 0.2-ml portion was taken to measure total drug recovered. Binding was calculated as follows: percent bound = 100 − ([disintegrations per minute of ultrafiltrate/disintegrations per minute recovered in protein mixture] × 100). Recovery was calculated as: percent drug recovered = (disintegrations per minute recovered/disintegrations per minute initial) × 100.

Ultrafiltration studies were performed by the method of Gerding et al. (7) as modified from Steinberg and Schachman (20).

A 6-ml amount of the drug-serum solution prepared as above was added to polyalamer tubes and centrifuged as 225,000 × *g* for 4.5 h at 24°C in a 50 Ti fixed-angle rotor (Beckman Instruments Inc.). After centrifugation, 0.2 ml of clear supernatant was removed for counting. Another 0.1 ml was used for protein determination by the method of Lowry et al. (11). Then the solution was thoroughly mixed, and a 0.2-ml portion was removed for counting. Binding was calculated as follows: percent drug bound = (disintegrations per minute of supernatant/disintegrations per minute in the mixed fluid) × 100; percent drug recovered = (disintegrations per minute after centrifugation/disintegrations per minute before centrifugation) × 100.

Lipid solubility. The apparent partition coefficients of the drugs in chloroform or octanol versus aqueous phosphate buffer (pH range 6.5 to 8.5 at 0.1 ionic strength) were determined by the method of Colaizzi and Klink (4). Solutions were mixed in a Dubnoff shaker (120 cycles/min) for 60 min. After separation of the layers by centrifugation, samples from each solvent were counted for radioactivity as described above.

Association with bacteria. *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and a patient isolate of *Proteus vulgaris* were grown in log phase by inoculating 20 ml of Trypticase soy broth overnight. Then these cultures were transferred to 180 ml of Trypticase soy broth for 4 more h. Cultures were centrifuged at 5,000 × *g* for 10 min and washed three times in medium C of Del Bene and Rogers (5). Bacterial suspensions were adjusted to an optical density of 0.6 at 625 μm, and viable counts were determined by plating on Trypticase soy agar. Cetocycline or tetracycline, at 0.84 μg and 0.5 μCi, were added to 0.1 ml of bacterial suspensions (approximately 2.5 × 10^{10} organisms per ml) and incubated at 37°C. Reactions were terminated by adding 9.9 ml of iced medium C at 5, 30, and 60 min. Bacterial-associated drug was determined by comparing counts in the mixed reaction mixture (total counts) to those in the supernatant (free counts) after centrifugation (15,000 × *g* for 15 min at 4°C). To assure that loss of counts from the supernatant represented pellet-associated counts, counts in the pellet were obtained. Greater than 93% of the counts removed from the supernatant could be accounted for in the pellet.

RESULTS

Comparison of antibacterial activity. The cumulative percentages of strains susceptible to cetocycline and tetracycline are shown in Table 1. Cetocycline was generally more active than tetracycline against *E. coli, Proteus, Citrobacter, Enterobacter, Providencia,* and *Serratia.* Activity of both drugs was about the same against *Klebsiella,* and neither drug was active against *Pseudomonas.* Tetracycline was more active against *S. aureus.* Distribution of tetracycline activity against *S. epidermidis* and nongroup A streptococci was biphasic, including *Streptococcus faecalis* strains (14) and *S. bovis* strains (4). In contrast, susceptibility to cetocycline was more uniform for these bacteria. All strains were inhibited by 6.2 μg/ml or less.

A total of 50 representative strains from the collection were assessed for MIC or MBC (Table 2). These studies indicate that for gram-negative organisms, the MBC was generally two to four times greater than the MIC for cetocycline, whereas the bactericidal effect of tetracycline, in most instances, was not observed at 50 μg/ml, the highest concentration tested. In contrast, tetracycline was most active against staphylococci and tended to be bactericidal at lower concentrations than cetocycline.

Effect of human serum on antibacterial activity. *S. aureus* ATCC 25923 was incubated in serial dilutions of both drugs in 50% fresh or heated human serum. Heating serum at 56°C

TABLE 1. *Comparative in vitro activity of cetocycline and tetracycline against gram-positive and gram-negative bacteria*

Organism (no. isolated)	MIC (μg/ml) to include cumulative %					
	Cetocycline			Tetracycline		
	50%	75%	90%	50%	75%	90%
P. mirabilis (34)	0.8	1.6	3.1	>50	>50	>50
P. morgagni (9)	0.8	0.8	1.6	>50	>50	>50
P. rettgeri (6)	1.6	3.1	6.2	50	>50	>50
P. vulgaris (1)	1.6			6.2		
Citrobacter (6)	0.8	3.1	3.1	1.6	3.1	>50
Enterobacter (6)	3.1	6.2	6.2	3.1	6.2	50
Providencia (6)	0.8	1.6	1.6	>50	>50	>50
Serratia (4)	1.6	1.6	3.1	6.2	50	>50
E. coli (79)	0.8	1.6	3.1	1.6	25	>50
Klebsiella (32)	1.6	3.1	3.1	1.6	3.1	12.5
Pseudomonas (38)	50	>50	>50	>50	>50	>50
S. aureus (30)	3.1	3.1	3.1	0.8	0.8	25
S. epidermidis (26)	1.6	3.1	3.1	0.8	>50	>50
Streptococci (17)	3.1	3.1	3.1	50	50	>50

TABLE 2. *Bacteriostatic and bactericidal activity of cetocycline and tetracycline against 50 bacterial strains*

Organism and (no. isolated)	Median value and (range)			
	MIC (μg/ml)		MBC (μg/ml)	
	Cetocycline	Tetracycline	Cetocycline	Tetracycline
E. coli (15)	3.1 (1.6-25)	6.2 (1.6->50)	6.2 (3.1->50)	>50 (12.5->50)
Klebsiella (6)	0.8 (0.2-6.2)	1.6 (0.8-6.2)	1.6 (0.4-25)	>50 (>50)
Enterobacter (5)	3.1 (1.6-6.2)	6.2 (3.1->50)	12.5 (6.2->50)	>50 (25->50)
Serratia (3)	3.1 (1.6-3.1)	>50 (6.2->50)	6.2 (6.2-12.5)	>50 (>50)
Providencia (3)	3.1 (1.6-3.1)	>50 (25->50)	3.1 (3.1-6.2)	>50 (>50)
Citrobacter (3)	3.1 (3.1-6.2)	1.6 (1.6-3.1)	6.2 (3.1-12.5)	>50 (3.1->50)
S. aureus (6)	3.1 (3.1-6.2)	0.4 (0.4-50)	25 (6.2-50)	1.6 (0.8-50)
S. epidermidis (6)	3.1 (1.6-3.1)	0.4 (0.4-0.8)	6.2 (3.1-25)	1.6 (0.4->50)
S. faecalis (5)	1.6 (1.6-3.1)	6.2 (6.2->50)	50 (6.2->50)	>50 (>50)

for 30 min did not alter the findings. The MBC of both drugs increased 64 times in the presence of serum. Cup plate assays with the same organism revealed a 50% decrease of cetocycline zone size diameters and a 26% decrease in tetracycline.

Serum binding. To fully characterize the inhibitory effect of serum on activity of the drugs, binding was measured by equilibrium dialysis, ultrafiltration, and ultracentrifugation. Several methods were used because of the fact that tetracyclines bind to glass and other surfaces (10).

Mean values for serum binding of the drugs in five or more duplicate experiments are shown in Table 3. Serum binding of cetocycline was greater than that of tetracycline, but the extent differed with each method. Binding of tetracycline was lowest by equilibrium dialysis, intermediate with ultracentrifugation, and highest with ultrafiltration. Binding of cetocycline was similar with ultrafiltration and ultracentrifugation, but lower with equilibrium dialysis. Increasing the drug concentration resulted in greater serum binding of tetracycline, but not of cetocycline.

Nonspecific drug loss was assessed by substituting buffer for serum (Table 4). Nonspecific binding probably represents binding to test tube walls, dialysis membranes, and constituents of the buffer. Nonspecific binding of tetracycline was less than 10% in all test systems. Cetocycline demonstrated higher nonspecific binding and minimal "negative" binding with ultrafiltration. After prolonged equilibrium dialysis, higher concentrations of cetocycline remained outside the dialysis bag as compared with inside and gave negative binding values. When cetocycline was initially placed in the dialysis bag, the reverse phenomenon was observed.

Lipid solubility. These studies were performed to fully understand the extensive serum binding of cetocycline and tetracycline and to predict their pharmacokinetic properties. Cetocycline was approximately 30 times more lipid soluble than tetracycline in octanol and 3 times more soluble in chloroform at pH 7.5 (Table 5). Both drugs were more lipid soluble at low pH. Tetracycline was more lipid soluble than cetocycline at pH 8.5 in chloroform.

Uptake by bacteria. To determine the relationship between in vitro antimicrobial activity of the drugs and their uptake by bacteria, three organisms were selected which differed in susceptibility to the agents (Table 6). Cetocycline uptake was significantly higher than tetracycline for all bacteria studied, despite their differences in in vitro antimicrobial activity. Only with *P. vulgaris*, which is highly resistant to tetracycline and susceptible to cetocycline, did higher uptake correspond with greater in vitro activity.

DISCUSSION

Cetocycline, an antibiotic closely related to the tetracyclines, is distinctive in that it is much more active than the tetracyclines against strains of *Proteus, Enterobacter, Providencia,* and *Serratia* in vitro. In addition, concentrations of cetocycline only two to four times higher than the MIC were bactericidal against sensitive gram-negative bacteria. Because the mode of action of cetocycline appears to be similar to tetracycline in inhibiting ribosomal protein synthesis (12), differences in activity may be due to other factors such as better penetration through the cell envelope or tighter binding to ribosomes. Minocycline, for example, is more active against some bacteria than other tetracyclines and is more avidly taken up by susceptible strains (5). However, cetocycline (unlike minocycline) is generally less active than tetracycline against staphylococci.

Lipid solubility of cetocycline was studied be-

TABLE 3. *Serum binding of tetracycline and cetocycline*

Drug	Concn (μg/ml)	Ultracentrifugation		Ultrafiltration		Equilibrium dialysis	
		% Bound[a]	% Recovery	% Bound[a]	% Recovery	% Bound[a]	% Recovery
Tetracycline	1	5.67 ± 4.2 (6)[b]	74	49 ± 8.1 (6)	81	18.8 ± 4.8 (6)	61
	5	26.3 ± 7.4 (6)	69	62.5 ± 11.9 (6)	69	24 ± 10.7 (5)	76
	10	34.8 ± 10.8 (6)	77	68.8 ± 8.7 (6)	71	25.5 ± 8.7 (6)	73
Cetocycline	1	82.2 ± 7.5 (6)	61	87.7 ± 1.8 (6)	79	69.4 ± 8.7 (5)	77
	5	85.6 ± 1.5 (5)	60	87.2 ± 2.6 (5)	85	70 ± 8.0 (6)	68
	10	85.7 ± 1.9 (6)	66	85.7 ± 4.3 (6)	84	68 ± 8.6 (6)	66

[a] Mean ± 1 standard deviation.
[b] Number in parentheses indicates number of duplicate samples.

TABLE 4. *Nonspecific binding of tetracycline and cetocycline as determined with a buffer control*

Drug	Concn (μg/ml)	Ultracentrifugation		Ultrafiltration		Equilibrium dialysis	
		% Nonspecific binding[a]	% Recovery	% Nonspecific binding[a]	% Recovery	% Nonspecific binding[a]	% Recovery
Tetracycline	1	5.2 ± 3.6 (6)[b]	99	5.7 ± 7.1 (6)	90	−1.2 ± 3.4 (6)	100
	5	5.5 ± 1.6 (6)	99	8.8 ± 5.1 (6)	87	−3.0 ± 3.5 (6)	98
	10	6.8 ± 1.7 (6)	98	7.2 ± 4.4 (6)	83	1.3 ± 4.3 (6)	95
Cetocycline	1	3.0 ± 3.5 (5)	73	16.6 ± 5.0 (5)	43	−10.0 ± 13.7 (6)	67
	5	6.4 ± 4.3 (5)	82	18.4 ± 8.8 (5)	37	−19.5 ± 7.9 (6)	64
	10	7.8 ± 4.8 (5)	79	13.6 ± 7.2 (5)	35	−35.3 ± 14.1 (6)	76

[a] Mean ± 1 standard deviation.
[b] Number in parentheses indicates number of duplicate samples.

TABLE 5. *Apparent partition coefficients of tetracycline and cetotetrine*

Drug	Concn (μg/ml)	Partition coefficient (mean ± standard deviation) of:					
		Chloroform at:			Octanol at:		
		pH 6.5	pH 7.5	pH 8.5	pH 6.5	pH 7.5	pH 8.5
Tetracycline	1	0.047 ± 0.008 (6)[a]	0.032 ± 0.006 (4)	0.020 ± 0.005 (6)	0.057 ± 0.009 (6)	0.040 ± 0.004 (4)	0.028 ± 0.002 (6)
	10	0.075 ± 0.013 (6)	0.042 ± 0.005 (4)	0.031 ± 0.006 (6)	0.068 ± 0.006 (6)	0.042 ± 0.004 (4)	0.027 ± 0.003 (6)
	50	0.092 ± 0.010 (6)	0.049 ± 0.006 (4)	0.029 ± 0.004 (6)	0.072 ± 0.005 (6)	0.048 ± 0.002 (4)	0.030 ± 0.003 (6)
Cetocycline	1	0.421 ± 0.130 (6)	0.088 ± 0.026 (8)	0.021 ± 0.003 (6)	3.363 ± 0.586 (6)	1.255 ± 0.220 (7)	0.310 ± 0.053 (6)
	10	0.621 ± 0.064 (6)	0.106 ± 0.019 (8)	0.021 ± 0.003 (6)	3.741 ± 0.501 (6)	1.345 ± 0.197 (8)	0.327 ± 0.052 (6)
	50	0.684 ± 0.111 (6)	0.135 ± 0.016 (8)	0.027 ± 0.004 (6)	4.294 ± 0.415 (6)	1.385 ± 0.129 (8)	0.366 ± 0.018 (6)

[a] Number in parentheses indicates number of experiments in duplicate.

cause of its pivotal role in determining serum protein binding, penetration into tissues, and uptake by bacteria (1). Octanol solubility at pH 7.5 was higher for cetocycline than for any other tetracycline analog studied by Colaizzi and Klink (4). Using their methods, we obtained similar results with tetracycline. Cetocycline, however, was more than two times more lipid soluble than doxycycline, the most lipid-soluble analog that they studied.

Lipid solubility of tetracycline analogs is highest when the compound is in dipolar ionic form (4). The pK_a values for tetracycline tricarbonylmethane group ($pK_{a1} = 3.3$), phenolic diketone group ($pK_{a2} = 7.7$), and dimethyl ammonia group ($pK_{a3} = 9.7$) (13) vary little from the corresponding groups in cetocycline ($pK_{a1} = 3.4$, $pK_{a2} = 7.6$, $pK_{a3} = 9.25$). However, cetocyline is unique among the tetracycline group in that it possesses a second aromatic methyl group that may increase lipid solubility. Interaction between the negative tricarbonylmethane group and the positive dimethyl ammonia group of tetracycline may cancel the charge at neutral pH, thus, increasing lipid solubility.

Lipid solubility is an attractive explanation for the greater activity of cetocycline and possibly its bactericidal action. Reynards et al. (15) found that as lipid solubility of tetracycline analog increased, antimicrobial activity increased in relatively resistant strains of *E. coli*. Lipid solubility may also account for the marked activity of minocycline against staphylococci (18). Our experiments only support this view in part because cetocycline was actually less active against *E. coli* and staphylococci. Only with *P. vulgaris*, a strain resistant to tetracycline and highly susceptible to cetocycline, could we correlate lipid solubility with uptake of the drug by the microorganism. The discrepancy may be partially due to the limitations of the method, because association of radiolabeled drug with the organism does not localize the exact site of drug penetration into the bacteria. Other authors have also noted that the quantity of drug associated with the bacteria does not always correlate with bacterial susceptibility (2, 15, 16).

Serum binding of cetocycline was considerably higher than that of tetracycline. Three different methods were used because of the problem of nonspecific binding of tetracyclines to surfaces that produces misleading results. Cetocycline did not reach equilibrium with the dialysis system (negative values for nonspecific binding) and showed high loss in the ultrafiltrate system (Table 4). Because the ultracentrifugation technique offered the only system uninterrupted by membranes, it is not surprising that it

TABLE 6. *Affinity of cetocycline and tetracycline for S. aureus, E. coli, and P. vulgaris*[a]

Organism	Drug	MIC (μg/ml)	Uptake (ng/10^8 bacteria ± standard deviation)	
			30 min	60 min
S. aureus	Tetracycline	0.4	62.2 ± 67.2 (9)[b]	37.7 ± 25.4 (9)
	Cetocycline	3.1	155.7 ± 105.3 (10)	165.0 ± 112.0 (10)
			$P < 0.05$[c]	$P < 0.01$
E. coli	Tetracycline	0.8	30.3 ± 22.1 (10)	43.2 ± 24.3 (10)
	Cetocycline	1.6	59.9 ± 19.4 (10)	66.7 ± 18.6 (10)
			$P < 0.02$	$P < 0.05$
P. vulgaris	Tetracycline	>50	28.2 ± 20 (24)	24.8 ± 22.9 (22)
	Cetocycline	0.8	113.3 ± 59 (16)	105.9 ± 51.8 (16)
			$P < 0.001$	$P < 0.001$

[a] Incubation was performed at 37°C with 2.5×10^{10} organisms per ml and 8.5 μg of drug per ml.
[b] Number in parentheses indicates number of determinations.
[c] P values determined by unpaired Student's t test comparing values for tetracycline to cetocycline at each time interval.

showed the least nonspecific binding and loss of drug. An association between lipid solubility and serum protein binding is well known for penicillins and tetracyclines (1, 9). Not all of the serum binding of cetocycline noted in the current study may be due to association with serum proteins. Kornguth, in our laboratory (unpublished data) found that serum ultrafiltrates also bind tetracyclines and that this is related to chelation with di- and trivalent cations. Other metals may also inactivate tetracyclines (19). Serum binding tends to decrease the proportion of free drug available for antimicrobial activity and cell penetration (8). However, this may be offset by increased antimicrobial activity. In addition, it is expected that high serum concentrations can be achieved because of delayed renal excretion and enterohepatic recirculation as described for doxycycline by Schach von Wittenau (17). In a study of the distribution of minocycline, doxycycline, tetracyline, and oxytetracycline in the body, Barza et al. (1) noted that lipophilicity correlated well with penetration through the blood-brain and blood-ocular barriers, and with a concentration gradient into bile. This correlation was higher with the octanol-water than with chloroform-water coefficients. Thus, it is expected that cetocycline will penetrate well into the brain and eye. Whether or not this leads to vertigo, a side effect reported with minocycline (6, 21), remains to be determined by clinical studies.

The major potential clinical use of cetocycline will probably be to treat urinary tract infections due to organisms frequently resistant to other agents. It is encouraging that all strains of *Proteus* tested, as well as *Enterobacter, Providencia,* and *Serratia,* were susceptible to cetocycline. Extrapolation to treatment of infections in humans, however, is premature until it can be shown that adequate concentrations are achieved in urine and efficacy is established by clinical tests. Furthermore, emergence of resistant strains is always a problem (3) and cannot be predicted from the current information about the drug.

ACKNOWLEDGMENTS

The authors are indebted to Ann Fisher for technical assistance in these studies. We also thank Cynthia Birch for editorial assistance on this manuscript.

These studies were supported by the Medical Research Service of the Veterans Administration and Abbott Laboratories.

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