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The Antibacterial Action of Erythromycin.* (19815)

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Erythromycin is a new antibiotic obtained from *Streptomyces erythreus*. Some features of the isolation and production of this antibiotic and of its physical, chemical and biologic properties were described briefly by McGuire *et al.*(1). They found erythromycin to be active *in vitro* against a wide variety of microorganisms, including strains of penicillin-sensitive and penicillin-resistant staphylococci, and of relatively low toxicity both for laboratory animals and in preliminary trials in patients. The prospect of a new agent that is active and nontoxic and which might prove to be effective against staphylococcal infections prompted us to undertake further studies on erythromycin. A preliminary clinical report has been published elsewhere(2) and the results of the laboratory findings are presented in this and the succeeding 2 papers. Some laboratory and clinical observations have also been presented by Heilman *et al.*(3).

Materials and methods. Erythromycin. A supply of "Ilotycin" (Erythromycin, Lilly) for clinical trial and of the purified crystalline material (890 μg/mg) for laboratory studies was furnished by the Lilly Research Laboratories through the courtesy of Dr. J. W. Smith. Stock solutions of the white crystalline powder were made either in broth or distilled water so as to contain 8 mg or 2 mg of the free base per ml; a small amount of ethyl alcohol was used to facilitate solution. Further dilutions of the antibiotic were made in broth unless otherwise indicated. *Bacterial Strains.* Most of the strains of organisms used in this study were recently isolated from infected material obtained from patients at the Boston City Hospital. Some of them were isolated and identified by Marion E. Lamb and A. Kathleen Daily in the Bacteriological Laboratory of the Mallory Institute of Pathology; the strains of gonococci were isolated by Helen W. Trousdale in the Genito-Urinary Clinic and most of the other organisms were isolated by Clare Wilcox and Marilyn K. Broderick to whom we are also indebted for technical assistance throughout this study. Most of the strains of specific groups and types of hemolytic streptococci were obtained from Drs. Rebecca Lancefield, Lowell A. Rantz and Harry A. Feldman. The strains of *Streptococcus mitis, sanguis* and *faecalis* from cases of bacterial endocarditis were obtained from Dr. Leo Loewe. In addition, a few laboratory strains used routinely for assay of other antibiotics were also included. For routine tests of sensitivity, the cultures used were obtained by picking individual colonies from the surface growth of culture plates; however, in the studies on the development of resistance and on the mechanism of action of the antibiotic, individual colonies were picked from agar pour plates of the cultures which had been so diluted as to contain only a few well segregated colonies. *Sensitivity Tests.* These were carried out with serial 2-fold dilutions of the

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TABLE I. Stability of Erythromycin in Broth or Serum at Various Temperatures.

Temp., °C	Solvent	Assayed erythromycin concentration, μg/ml — Days of storage: 0	1	2	4	7	14	21	28	44	56
−25, 5	Broth	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000
22–30	"	2000	2000	2000	2000	1000	2000	1000	1000	1000	1000
37	"	2000	2000	2000	1000	1000	1000	1000	500	250	125
−25	"	2	2	2	2	2	2	2	1	2	2
5, 22–30	"	2	2	2	2	1	1	1	1	1	1
37	"	2	2	2	2	1	.5	.5	.25	.25	.13
−25, 5	Serum*	2000	2000	2000	2000	2000	2000	2000	2000		
37	"	2000	2000	2000	1000	1000	1000	500	250		
−25, 5	"	2	2	2	2	2	2	2	2		
37	"	2	2	2	1	.5	.5	.25	.13		

Final pH of the 2000 μg solution in broth was 7.7, that of the 2 μg solution was 7.5. *Sarcina lutea* in a dilution of 10^{-4} was used as the assay organism.

* 50% pooled human serums.

TABLE II. Effect of Heat and Filtration on Activity of Erythromycin in Broth.

Treatment	Temp., °C	Duration	Assayed conc. of erythromycin, μg/ml — Before	After	Before	After
Heated	60	5 min., 15	200	100	2	1
		1 hr, 2	"	50	"	"
Heated	100	5 min., 15	"	"	"	.5
		1 hr, 2	"	12.5	"	.125
Seitz filtered			"	50	"	1
Berkefeld filtered			"	"	"	"
Sintered glass filtered			"	100*	"	" *
None—control			"	200	"	2

Streptococcus 98 diluted 10^{-4} used for assays in broth.

* Partial inhibition in twice this concentration.

antibiotic, either in broth (Brain-Heart Infusion Broth, Difco, pH 7.4±) or in agar (Heart-Infusion Agar, Difco, pH 7.4±). Defibrinated horse blood, 0.5%, was added to the broth to serve as an indicator of growth and 10% was added to the agar for use with hemolytic or fastidious organisms. The inoculum in the broth-dilution test was such as to make a final dilution of 10^{-4} of the seed culture; in the agar-plate dilution test a 2 mm loopful of the undiluted culture was used. The sensitivity of a strain is expressed as the final concentration of antibiotic in the medium that completely inhibited growth in 18-24 hours as judged by visible turbidity or hemolysis in the broth or by the appearance of growth or hemolysis on the agar plates. Growth inhibition in broth was verified by subculture on antibiotic-free agar.

Observations on Antibacterial Properties of Erythromycin. Effect of Storage. Solutions containing 2000 and 2 μg/ml in broth were stored as follows: a) at −25°C in a "deep freeze," b) at 5°C in a refrigerator, c) at 22-30°C on a laboratory shelf and d) at 37°C in an incubator. Similar solutions in 50% pooled human serum were also stored in like manner, but omitting room temperature. As shown in Table I, all of the solutions retained their activity remarkably well at −25°C throughout the periods of observation. The same was true at 5°C, except for a detectable

TABLE III. Effect of pH of Medium on Sensitivity of Bacteria to Erythromycin.

pH of broth	Min complete inhibiting conc. of erythromycin, μg/ml *Strep.* 98	*S. lutea*
5.58	1.6	.8
6.05	.8	.4
6.51	.2	.1
7.08	.04	.04
7.46	.02	.01
8.07	.01	.003
8.53	.003	.001
	Growth without antibiotic	
5.58	+	++
6.05	++	+++
6.51		
7.08	+++	+++
7.46		
8.07	+++	++
8.53	++	+

drop in activity of the 2 μg solution in broth by the end of the first week without further deterioration thereafter. At room temperature there was a similar loss of activity in broth at both concentrations, while at 37°C there was progressive deterioration in activity of all the solutions after 4 or 7 days.

Effect of heating. As shown in Table II, there was some loss of activity detected even after 5 minutes at 60°C; the loss appeared to be greater from the higher concentration and after longer exposures, particularly at 100°C.

Effect of Filtration. (lower part of Table II). Passage through a Seitz filter pad (Hercules, type ST, size L3) or through a Berkefeld filter entailed a detectable loss of activity from a concentration of 2 μg and a 75% loss from a 200 μg/ml solution in broth. In solutions passed through sintered glass filters (Corning UF) there was also a slight loss. All of the filters, except the Seitz filter pads, had previously been used several times for other materials.

Effect of pH. The erythromycin sensitivity of the test strains *Strep.* 98 and *S. lutea* were determined in broth adjusted to various pH levels ranging from about 5.6 to 8.5. As shown in Table III, the sensitivity of both strains increased progressively with increasing alkalinity of the medium; the increase was roughly 10-fold for each pH unit. Growth at the extreme pH values was poor even without antibiotic, but the inhibition by erythromycin was clearly discernible. This effect of pH is similar to that observed with streptomycin(4) and is the reverse of that noted with aureomycin(5).

Effect of Various Substances. The following substances, in the final concentrations indicated, were used in heart infusion broth for assay of the erythromycin sensitivity of *Bacillus cereus,* No. 5:

Sodium chloride Dextrose	M/100, M/10, 1M
Sodium thioglycollate Cysteine hydrochloride Semicarbazide Urea Glutamic acid	M/1000, M/100, M/10
Para-aminobenzoic acid	M/1000, M/500, M/100
Pteroylglutamic acid	M/1000, M/100, M/50

The following substances were similarly used with *Streptococcus* 98 as the test organism: the sodium salts of citric, pyruvic, acetic, lactic, fumaric and succinic acid, each in concentrations of M/100, M/10 and 1M. Penicillinase, 1000, 100, and 10 units, was also used with this organism. Pooled human serum was added to broth in concentrations of 12.5, 25, and 50% and assayed with *S. lutea* as the test strain. None of these substances, in the concentrations used, had any significant effect on the antibiotic activity of erythromycin against these respective test organisms in the crude test here employed.

Effect of Reduced Oxygen Tension. Two sets of blood-agar plates containing serial 2-fold dilutions of erythromycin were prepared and segments of each were inoculated with 2 strains of *Streptococcus* (Nos. C203 and 98), and one each of *S. lutea,* pneumococcus type 3, *Streptococcus viridans,* enterococcus and staphylococcus (No. 195). One set of these plates was then incubated at 37°C on an open shelf; the other was placed in a tightly sealed jar from which the oxygen was partially removed and CO_2 tension increased by burning a candle inside to extinction and the jar was then placed in the same incubator. The end-points of inhibition by erythromycin were identical for each organism in the 2 sets of plates. However, on the plates with the highest concentrations of antibiotic on which growth still occurred, that growth in the case

of 4 of the strains was heavier after 24 hours in the candle jar than on the open shelf. End-points of inhibition by erythromycin were also found to be the same in brain heart infusion broth and in thioglycollate broth.

Erythromycinase. An attempt was made to determine whether erythromycin-inhibiting substances are produced during growth by organisms which are relatively resistant to that agent. In one series of studies the method used was essentially the same as the modified Gots test for penicillinase(6). A strain of *S. lutea* was employed; its growth was completely inhibited on the surface of agar containing 0.02 μg but not by 0.01 μg of erythromycin per ml. Plates were poured with agar containing both a 1:100 dilution of a fully grown culture of this strain and erythromycin in a final concentration of 0.02 μg/ml. The agar was allowed to harden rapidly in the cold and a number of strains of organisms were streaked radially on segments of these plates. The following organisms were used: 13 strains of *Pseudomonas aeruginosa*, 6 of *Aerobacter aerogenes*, 5 of *Klebsiella pneumoniae*, 7 of *Escherichia coli*, 4 of *Proteus vulgaris*, 2 species of *Salmonella* and 2 species of *Shigella*. In plate-sensitivity tests, all of these strains grew well on agar containing 200 μg/ml of erythromycin except the strains of *Salmonella* and *Shigella*, growth of the former being inhibited by 200, and of the latter by 100 μg ml. All of the strains grew rapidly and luxuriantly on the surface of the agar containing the Sarcina and erythromycin. In no instance, however, was growth of colonies of Sarcina made out within the agar in the vicinity of the surface growths, the underlying and surrounding agar remaining perfectly clear as did the rest of the agar. This was taken to indicate that no erythromycin-inhibiting substances had diffused from the surface growth of any of the strains into the medium, at least before the erythromycin contained in that agar had either killed or completely inhibited the growth of the Sarcina. In a second type of experiment, there was no indication that the growth of *P. vulgaris* or *E. coli* in solutions of erythromycin for periods up to 72 hours had inactivated any of the antibiotic.

TABLE IV. Effect of Size of Inoculum on Erythromycin Sensitivity.

Size of inoculum*	M.I.C.,† μg/ml Broth dilution	M.I.C.,† μg/ml Agar-plate dilution	Growth without antibiotic: Broth	Growth without antibiotic: Agar
		Streptococcus 98		
Undiluted	—	.1	—	+++
10^{-1}	1.6	.04	+++	+++
10^{-2}	.2	.02	+++	+++
10^{-3}	.04	.02	+++	+++
10^{-4}	.02	.02	+++	+++
10^{-5}	.02	.01	+++	5 colonies
10^{-6}	.02	—	+++	—
		Sarcina lutea		
Undiluted	—	.02	—	+++
10^{-1}	.04	.02	+++	+++
10^{-2}	.04	.02	+++	+++
10^{-3}	.04	.02	+++	++
10^{-4}	.02	.01	+++	+
10^{-5}	.02	<.005	+++	1 colony

* Final dilution of culture in the broth-dilution test and the dilution from which a 2 mm loopful was streaked on each plate in the agar-plate dilution test. Pour plate counts of the original 18-hr cultures yielded approximately 10^8 colonies per ml of *Strep.* 98 and 2×10^7 colonies per ml of *S. lutea*.

† Minimum complete inhibiting concentration.

— = Not done.

Effect of Size of Inoculum. *Streptococcus* 98 and a strain of *S. lutea* were tested for sensitivity to erythromycin by both the broth- and the agar-dilution methods using inocula of varying sizes. The results are shown in Table IV. In these tests the strains appeared to be more sensitive when a small inoculum was used; the differences were greater with *Strep.* 98 than with *S. lutea*. However, there were considerable ranges of concentrations of culture in each test and with each strain over which the same end-points were obtained. Similar effects have been noted with other antibiotics(7). From a practical point of view, these results indicate that when tests for sensitivity to erythromycin are carried out in the routine manner employed here, that is, with the culture diluted to 10^{-4} in broth, or used undiluted or up to 1:10 on agar, the value obtained for the sensitivity of an organism may be the same by the two tests (as in the case of *S. lutea*) or the plate-dilution value may be higher (as with Strep. 98).

Comparison of Agar- and Broth-Dilution Methods for Determining Sensitivity to Erythromycin. It has been shown(7) that values for sensitivity of any given strain to a number

TABLE V. Comparison of Results of Erythromycin Sensitivity Tests Done by Broth-Dilution and Agar-Dilution Methods.

Organism	Min inhibiting conc., μg/ml — On agar — Complete	On agar — Partial	Complete / Partial	In broth — Complete	In broth — Partial	Complete / Partial	M.I.C. on agar / M.I.C. in broth — Complete	Partial
Streptococcus, C203	.04	.02	2	.02	.01*	2	2	2
98	.04	.02	2	.02	.01*	2	2	2
Bacillus cereus, 5	.4	.1	4	.2	.1	2	2	1
S. lutea	.02	.01	2	.02	.02	1	1	½
Staph. aureus, 195	.4	.2	2	.4	.2	2	1	1
192	.4	.2	2	.4	.2	2	1	1
193	.8	.2	4	.8	.2	4	1	1
194	.8	.2	4	.4	.1	4	2	2
197	.8	.2	4	.4	.1	4	2	2
Kl. pneumoniae, T	100	12.5	8	25	25	1	4	½
E. coli, MacLeod	100	12.5	8	12.5	6.3*	2	8	2
14	100	12.5	8	6.3*	6.3	2	16	2
15	100	12.5	8	6.3	6.3	2	16	2
Ps. aeruginosa, 5, 6, 8	>200	200		>200	>200			

All these results were recorded at 24 hr; they were identical after 48 hr in every instance when done by the agar plate method.

* In the tests done in broth, however, the values indicated by the asterisks (*) were twice as high after 48 hr.

of antibiotics may vary within wide limits depending on the details of the method used. The standard strains employed in antibiotic assays in this laboratory and a few additional strains were tested by both methods simultaneously, the same cultures and dilutions of antibiotic being employed for all tests. The results are shown in Table V. In these tests the value for "partial" inhibition indicates the minimum concentration of antibiotic with which there was significantly less growth than that observed in the corresponding culture without antibiotic. Only the readings made after 24 hours of incubation are shown in Table V, but in the case of the tests done on agar, identical readings were obtained at 48 hours in every instance. The same was true of the values for complete inhibition in broth except for one strain; in the tests in broth, however, there were a few strains in which the minimum concentration of erythromycin showing partial inhibition was one dilution higher at 48 hours than that recorded at 24 hours. The ratio of complete to partial inhibiting concentrations may be taken as an indication of the sharpness of the end-point of the test. In broth, this ratio was usually 2 (*i.e.*, only one 2-fold dilution tube showed partial inhibition), although for 3 of the strains of staphylococcus this ratio was 4 (*i.e.*, 2 tubes showed partial inhibition). On agar, the results were essentially the same except that the strains of *E. coli* and that of *Kl. pneumoniae* showed an 8-fold difference. This may be due to the fact that minor degrees of growth are more readily discernible with the strains on the agar plates than by grossly visible turbidity in broth. As a result, the values for sensitivity obtained for these coliform organisms, as measured by complete inhibiting concentrations were 4 to 16 times higher by the agar method than by the broth method. For the Gram-positive strains, the values obtained by both methods were either the same or varied by only 1 dilution regardless of whether partial or complete inhibition was chosen for the end-point. Because of its greater simplicity, the agar plate dilution method was, therefore, adopted for tests involving large numbers of cultures, it being appreciated that for coliform organisms (and possibly for others, as noted in the section dealing with size of inoculum) the values for sensitivity by this method may be appreciably higher than those obtainable in the broth-dilution method.

Sensitivity of Pathogenic Bacteria to Erythromycin. Tests for sensitivity to erythromycin were done by the agar plate-dilution method on 1037 strains of pathogenic bacteria,

TABLE VI. Sensitivity of 1037 Strains of Bacteria to Erythromycin.

A. "Sensitive" organisms	No. tested	Min complete inhibiting conc./M.I.C., μg/ml 3.1	1.6	.8	.4	.2	.1	.04	.02	.01	M.I.C. for ⅔ of strains, μg/ml
Pneumococcus, various types	64						6	29	27	2	.04
Streptococcus hemolyticus:											
Group A	58					1	7	30	20		.04
Group B	14				3	7	2	2			.2
Group C	21			2	4	8	5	2			.2
Group D	30	6	7	4	3	2	3	2	3		1.6
Group E	2						1	1			—
Group F	4							2	2		.04
Group G	20					6	7	4	2	1	.1
Endocarditis strains:											
Alpha streptococcus	12	1	2	1	1	2	2	1	2		.4
Streptococcus mitis	3	2					1				3.1
sanguis	7						2	2	3		.04
faecalis	4	2	2								3.1
Staphylococcus aureus	640		6	28	529	60	11	1	2	3	.4
albus	10	1	3	3	2	1					1.6
Corynebacterium diphtheriae	14	1	4	4	4	1					1.6
Neisseria gonorrhoea	10				3	4	1	2			.2
meningitidis	8		1	1	3	3					.4
Hemophilus influenzae	18	3	9	5	1						1.6
hemolyticus	10	2	4	2	2						1.6

B. "Resistant" organisms		M.I.C., μg/ml >200	200	100	50	
Escherichia coli	17		5	8	4	100
Aerobacter aerogenes	12	2	10			200
Klebsiella pneumoniae	12	9	3			>200
Proteus vulgaris	8	8				>200
Pseudomonas aeruginosa	29	29				>200
Shigella flexneri	2	2				—
dysenteriae	1			1		—
Salmonella typhosa	4	2	1	1		>200
typhimurium	2	2				—
derby	1	1				—

most of them recently isolated from human sources. The results are shown in Table VI. All of the strains of Gram-positive cocci, *Neisseria* and *Hemophilus* were completely inhibited by 3.1 μg/ml or less and are listed as "sensitive"; the strains of coliform and enteric organisms all required 50 μg/ml or more and are listed as "resistant." The most sensitive were the pneumococci and group A hemolytic streptococci, while the most resistant of the organisms tested were the strains of *Proteus* and *Pseudomonas*. There were variations in sensitivity among strains of the same species. In the case of most of the species studied, however, these variations were confined to a 10-fold range of concentrations with more than three-fourths of the strains of each species being inhibited within a 4-fold range. The most divergent in sensitivity were the strains of group D streptococci and alpha hemolytic streptococci which were inhibited by concentrations varying from 0.02 to 3.1 μg/ml and those of *Staphylococcus aureus* which varied in sensitivity from 0.01 to 1.6 μg/ml. In the case of the streptococci, however, the strains were irregularly distributed throughout this range, whereas by far the greatest majority of the strains of *Staph. aureus* (87%) were inhibited by 0.4 μg/ml and 96% of them were inhibited by 0.2-0.8 μg/ml.

Comparison with Other Antibiotics. The great majority of the strains listed in Table VI have also been tested for sensitivity to the other antibiotics that are in common use, particularly to penicillin, streptomycin, aureo-

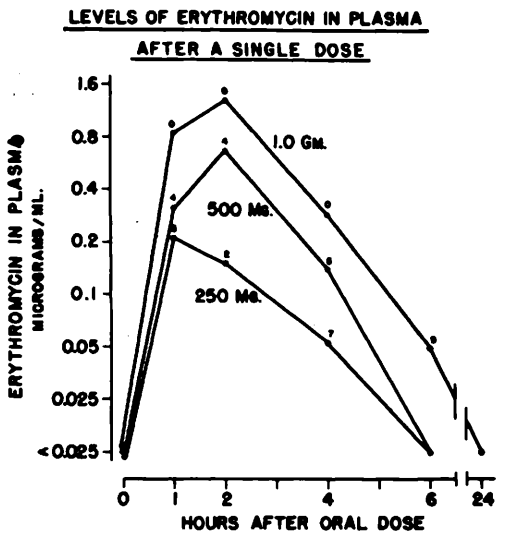

FIG. 1. Each point represents the mean for 15 samples. No. above each point indicates samples with <.025 γ/ml.

mycin, chloramphenicol and terramycin. On a weight basis, erythromycin most closely resembled penicillin in its activity; it was appreciably more active against Gram-positive organisms and less active against coliform and enteric bacilli than were the broad-spectrum antibiotics or streptomycin. Erythromycin was comparable with all of these agents in its inhibition of strains of *Hemophilus*. The majority of the strains of *Staph. aureus* included in Table VI were markedly resistant to penicillin, but erythromycin was equally active against both the penicillin-sensitive and the penicillin-resistant strains. Streptomycin-resistant organisms were also included in the tests and these likewise were as sensitive to erythromycin as were streptomycin-sensitive strains of the same species.

Absorption and Excretion. Single doses of 250 mg, 500 mg and 1.0 g were given by mouth to adults who had no recent antimicrobial therapy. Each dose was given to 15 subjects of varying ages and weights but selected so that the groups receiving the different doses were comparable in these respects. Specimens of citrated venous blood were collected before and at 1, 2, 4, 6 and 24 hours after the dose and the plasma was assayed for erythromycin by the 2-fold tube-dilution method, using *Strep.* 98 as the assay organism. The minimum assayable concentration in these tests was 0.025 μg/ml. None of the initial plasma specimens showed any erythromycin activity. The levels obtained after the single doses varied considerably in the individual patients, but in general higher concentrations were obtained more frequently after the larger doses. One subject who received 250 mg and 2 who got 500 mg failed to yield assayable erythromycin activity in any of the specimens and another patient who was given the smaller dose had a minimum demonstrable level only in the 2-hour specimen. In the specimens obtained at 6 hours, there was no demonstrable erythromycin in any specimen from patients who received 250 or 500 mg nor from 9 of those who received 1.0 g.

The maximum plasma concentration of erythromycin in each patient was found either at 1 or 2 hours after the dose; it was found in the 1-hour specimen most frequently after the smallest dose and in the 2-hour specimen after the larger doses. The mean concentrations of erythromycin at various intervals after each of the doses are charted in Fig. 1, which also shows the numbers of subjects who failed to yield assayable levels.

Plasma concentrations of erythromycin were also studied in patients receiving repeated doses of the antibiotic for therapy of infections. The results are shown in Table VII. Although some specimens contained no demonstrable antibiotic after repeated doses of 100 or 125 mg, in patients treated with doses of 200 mg or more every 3 or 4 hours erythromycin activity could be demonstrated in the plasma throughout the intervals between the doses. In general the concentrations varied with the dose and, in most patients, with the interval after the dose. Assays were also made of erythromycin in the urine of patients under treatment with this agent. In 6 patients who received a single dose of 0.25-1.0 g, the urine collected during the ensuing 24 hours showed no demonstrable activity in 5; a mean concentration of 0.04 μg/ml and a total of 60 μg was found in the 24 hour specimen from the remaining patient who had received 1.0 g. In several subjects who were receiving 100-200 mg every 4 hours, no erythromycin activity was demonstrable in the urine during the first

TABLE VII. Concentrations of Erythromycin in Plasma of Patients During Treatment.

Dose	Day of Rx	Erythromycin in plasma, μg/ml —Hr after dose— 1	2	3	4
100 mg every 3 hr	1	0*	0	0	
	2	0	0	0	
	2	.4	3.1	.4	
	3	0	0	0	
	3	0	.2	0	
	4	0	.4	0	
	6	0	.4	0	
	6	0	.4	0	
125 mg every 3 hr	3	.1	0	0	
	3	.4	.2	0	
	4	0	.2	0	
200 mg every 3 hr	3	0	.2	.1	
	3	.2	.2	.2	
	5	.2	.4	.2	
250 mg every 4 hr	3	.8	.8	.8	.2
	4	3.1	.4	.2	.1
	4	6.3	3.1	.8	.2
	5	1.6	.8	.4	.2
	5	.8	3.1	.8	.4
	6	3.1	1.6	.2	.2
	6	.8	1.6	1.6	.2
500 mg every 3 hr	5	12.5	6.3	3.1	
	6	25	6.3	3.1	
	6	6.3	3.1	1.6	
	7	3.1	3.1	1.6	
	10	12.5	6.3	6.3	
	11	6.3	3.1	3.1	
	17	6.3	25	3.1	

* 0 = <.025 μg/ml.

4 days of therapy. In those receiving 500 mg every 3 hours concentrations ranging from 50-800 μg ml of urine were found in different specimens. Assays of total 24-hour urinary outputs were made at various intervals during the course of treatment of a number of patients; amounts of erythromycin varying from 0.02-15% of the daily amount ingested were recovered in different subjects. The greatest amounts were recovered during the second and third week of therapy from patients receiving doses of 500 mg every 3 hours. In a patient with staphylococcal meningitis under treatment with 500 mg of erythromycin every 3 hours, 5 specimens of spinal fluid were obtained and assayed at different times; concentrations varying from 0.1 to 0.8 μg/ml were found. The plasma obtained simultaneously was found to have 0.4 to 12.5 μg/ml, or from 4 to 16 times the concentration of the corresponding spinal fluid specimens.

Discussion. The data presented indicate that erythromycin has many of the physical, biochemical and biological properties of a useful antibiotic. The range of bacteria against which this agent is active is very similar to that of penicillin and is also embraced by the "broad-spectrum" antibiotics. In preliminary clinical trials(1-3) erythromycin appears to be effective against simple infections with Gram-positive cocci. However, the ready emergence of highly resistant variants among some of the organisms in the present studies and the clinical counterpart already observed in cases of bacterial endocarditis (1, 3) indicate that erythromycin will not be effective against all infections with these and other organisms, even if they do prove to be highly sensitive before treatment is begun. Failures may, therefore, be expected in situations where reasonably rapid sterilization of the lesion is not achieved.

However, there are many circumstances, such as hypersensitivity or untoward reactions of the patient to other effective antimicrobial agents, or resistance of the specific inciting strains to the other agents, in which erythromycin may prove to be very useful. Moreover, studies now in progress in this laboratory (by Drs. S. S. Wright and E. M. Purcell) indicate that the emergence of resistance to both erythromycin and streptomycin can be greatly delayed in organisms which are originally sensitive to both of these agents and are repeatedly subcultured in graded concentrations of both of them used in combination. These findings suggest that judiciously chosen and appropriately applied therapy with other antimicrobial substances in combination with erythromycin may extend the usefulness of the latter in the treatment of some important infections, for example those due to staphylococci which are resistant to many other agents.

Summary and Conclusions. The results of studies on some properties of erythromycin which may be important in its clinical and laboratory applications have been reported. 1. Erythromycin solutions retained their activity after prolonged storage in the cold or in the frozen state but showed progressive deterioration after several days at room or incubator temperatures or after brief exposures

to 60°C or higher. 2. Filtration of erythromycin through bacterial filters entailed the loss of some activity. 3. The antibacterial action of erythromycin increased progressively with increasing alkalinity of the culture medium, within the pH range of bacterial growth. 4. Many substances which may affect the action of other antimicrobial agents had no important effect on the action of erythromycin. 5. Erythromycin inhibiting substances could not be demonstrated in cultures of erythromycin-resistant bacteria. 6. The size of the inoculum affected the results of sensitivity tests with erythromycin, but there was considerable tolerance within the range of inocula used in clinical testing. 7. The broth-dilution and agar-plate dilution methods generally gave comparable results in tests for sensitivity of bacteria to erythromycin but the values obtained by the agar method often were higher, particularly with some coliform organisms. 8. Tests done on more than 1000 bacterial strains, most of them recently isolated from patients, indicated that erythromycin was most active against the Gram-positive cocci and was quite active against strains of *Neisseria,* diphtheria bacilli and *Hemophilus,* but for practical purposes it could be considered inactive against most coliform and enteric bacilli. 9. Concentrations of erythromycin in plasma after single oral doses varied widely but in general were proportional to the dose. Maximum concentrations were found 1 or 2 hours after a dose, and no activity was demonstrated at 6 hours except following doses of 1.0 g. Significant concentrations were maintained in the plasma with oral doses of 250 mg or more every 3 or 4 hours. 10. The amounts of erythromycin activity recovered in the urine appeared to be small and erratic after ingestion of single doses or after repeated small doses, particularly early in the course of therapy; however, on continuous therapy with divided doses, up to 15% of the amount ingested daily could be demonstrated in active form in the urine. Results of studies on the mode of action of erythromycin and on the resistance of bacteria to that agent are presented in the succeeding papers.

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Resistance of Bacteria to Erythromycin.* (19816)

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In the course of studies on some of the antibiotic and other important biologic properties of erythromycin, a number of which were presented in a previous paper(1), observations were also made on the occurrence or development of resistance of bacteria to erythromycin. These included a search for resistant variants in cultures of "sensitive" strains, with and without exposures to the antibiotic. The development of resistance in bacteria during treatment of patients was also observed. Some of the details of these observations are presented here. The methods and materials

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