Participation of Erythromycin and Carbomycin in Combined Antibiotic Action *in vitro*.* (20452)

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Recently two new antimicrobial agents, erythromycin and carbomycin, have appeared on the market(1,2). Early work on these drugs has indicated that their antimicrobial spectrum is very similar(3), that they give nearly complete cross-resistance(4), and that drug-resistant variants emerge quite rapidly from bacterial populations(3). It has been pointed out that simultaneous exposure of bacterial populations to erythromycin and either penicillin or streptomycin greatly diminishes the frequency of such resistant variants(5). For this reason it appeared likely that these new drugs might often be used in combinations with other antimicrobial agents. It was of interest, therefore, to examine the behavior of erythromycin and carbomycin when combined with other drugs, particularly with regard to the occurrence of synergism or antagonism.

Earlier reports from this laboratory have dealt with combined antibiotic action as manifested by the rate of bactericidal action(6-8). From the standpoint of bactericidal rate *in vitro* and *in vivo* the following scheme of combined antibiotic action has been proposed(9): Antibiotics fall into 2 broad groups: I. penicillin, streptomycin, bacitracin, neomycin. II. aureomycin, chloramphenicol, terramycin. Members of Group I are often synergistic with one another, sometimes indifferent, never antagonistic. Members of Group II are neither synergistic nor antagonistic with one another but may show simple additive effects. A combination of Group I with a Group II antibiotic may result in either indifference, synergism, or antagonism, depending in part on the manifest behavior of the microorganism toward the individual drugs. This scheme has proved useful in planning experiments and may find application in combined antibiotic therapy in clinical medicine. Hence it seemed advisable to establish the position of the new antibiotics in this tentative scheme.

Materials and methods. The test organisms included *Klebsiella pneumoniae* (A-D), *Micrococcus pyogenes var. aureus* (Heatley), and *Streptococcus fecalis* (No. 16), all of which had been used in establishing the original division of the antibiotics into 2 groups(7). In addition, 3 strains of *M. pyogenes var. aureus* (Co, Da, and Sh) recently isolated from patients were tested. Commercial preparations of crystalline sodium penicillin G, streptomycin sulfate, and bacitracin were used. Terramycin hydrochloride was obtained from Dr. G. L. Hobby, chloramphenicol from Dr. G. Rieveschl, erythromycin from Dr. L. E. Josselyn, and carbomycin from Dr. Frederick C. Fink. The drugs were dissolved in 0.85% sodium chloride and final dilutions were made in broth immediately before use. Proteose No. 3 broth containing the diluted antibiotics was inoculated with an 18-hour broth culture of the test bacteria to give a concentration of 10^7 to 10^8 organisms per ml in a total volume of 15 ml. Samples of 0.5 ml were removed at intervals during incubation at 37°C and the number of viable bacteria was estimated by plate counts on Proteose No. 3 agar (Difco). Details of the method and its interpretation have been described(10).

Results. When acting alone, erythromycin and carbomycin behaved in a similar manner except that a larger dose of the latter was often required for an antibacterial effect. Both drugs in adequate doses exerted a bactericidal effect during the first 24 hours of action but some bacteria usually survived and subsequently multiplied.

When bacteriostatic or slowly bactericidal doses of either erythromycin or carbomycin were combined with a rapidly bactericidal dose of penicillin, these new drugs sometimes

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TABLE I. *In Vitro* Action of Erythromycin and Carbomycin with Penicillin, Streptomycin, or Bacitracin, Estimated by Plate Counts of Viable Bacteria.

Test organism	Drug and amt (μg/ml) (bacitracin in units/ml)	No. of viable bacteria/ml — Hr after inoculation: 0	7	24	32-48	Combined effect
Micrococcus pyogenes var. aureus (Heatley)	None	6×10^6	8×10^7	6×10^8	10^9	
	Penicillin .3		2×10^4	10^1	10^1	
	Erythromycin 2.0		2×10^6	8×10^4	6×10^4	
	Pen .3 + erythro 2.0		2×10^6	3×10^4	5×10^8	Antagonism
	Carbomycin 2.0		3×10^5	8×10^4	5×10^4	
	Pen .3 + carbo 2.0		5×10^5	2×10^4	5×10^8	"
Klebsiella pneumoniae A-D	None	10^7	4×10^8	6×10^7	10^8	
	Penicillin 6.0		4×10^4	10^1	3×10^1	
	Erythromycin 10.0		6×10^5	3×10^6	10^8	
	Pen 6.0 + erythro 10.0		5×10^5	2×10^3	8×10^2	Antagonism
	Streptomycin 5.0		3×10^3	3×10^7	10^8	
	Strepto 5.0 + erythro 10.0		4×10^2	10^1	10^1	Synergism*
Micrococcus pyogenes var. aureus (Co)	None	3×10^7	2×10^8	5×10^8	10^9	
	Bacitracin 1.0		6×10^7	5×10^8	8×10^8	
	Carbomycin 1.0		9×10^6	5×10^6	7×10^7	
	Baci 1.0 + carbo 1.0		5×10^5	2×10^2	10^2	Synergism*
Micrococcus pyogenes var. aureus (Sh)	None	2×10^7	3×10^8	6×10^8	10^9	
	Bacitracin 10.0		3×10^6	4×10^4	4×10^8	
	Erythromycin .2		3×10^7	4×10^7	7×10^7	
	Baci 5.0 + erythro .2		5×10^5	10^2	4×10^1	Synergism*

* Synergism denotes a bactericidal effect *greater* than that obtained with 2-5 times larger concentrations of each single drug, used alone.

TABLE II. Combined *In Vitro* Action of Erythromycin and Carbomycin with Penicillin or Bacitracin.

Antibiotic combination	Test organisms — *M. pyogenes var. aureus* strain: H	Da	Sh	Co	*K. pneumoniae* strain A-D	*S. fecalis* strain #1-6
P + T	Antag.	Indiff.	Indiff.		Antag.	Antag.
P + E	"	"	"		"	"
P + C	"	"	"		"	"
S + E				Indiff.	Synerg.	
S + C				Addit.	Addit.	
B + T	Synerg.	Addit.	Synerg.	"		
B + E	Indiff.	"	"	"		
B + C	"	"		Synerg.		

Indifference = effect equal to that of the more active drug alone. Antagonism = effect less than that of the more active drug alone. Addition = simple algebraic summation. Synergism = effect greater than simple algebraic summation of single drug effects. Blank = not tested.
P = penicillin; B = bacitracin; E = erythromycin; C = carbomycin; T = terramycin; S = streptomycin.

reduced the bactericidal rate of the penicillin. Both erythromycin and carbomycin interfered with the bactericidal effect of penicillin acting on the test strains of *K. pneumoniae, S. fecalis,* and *M. pyogenes var. aureus* (Heatley) (Tables I and II). This antagonism was similar to that previously shown for chloramphenicol, aureomycin, and terramycin using the same test organisms(8,10,11). On the other hand, against *M. pyogenes var. aureus* (Sh and Co), erythromycin was synergistic with the Group I drug, bacitracin. Combinations of bacteriostatic doses of the new drugs with bacteriostatic doses of Group I drugs often showed an additive effect. A similar relationship has been established for bacteriostatic amounts of Group I with Group II drugs(8). Hence, erythromycin and carbomycin behaved in a manner resembling Group II antibiotics (Table II).

Combinations of erythromycin with carbomycin had only negligible additive effects. Mixtures of either chloramphenicol or terramycin with these new agents resulted in a

combined effect no greater than that obtained by increasing proportionately the dose of either drug used singly. In this regard also erythromycin and carbomycin behaved like members of Group II.

Antibiotics of Group II are, to some extent, interchangeable in combinations. Erythromycin or carbomycin, however, could not always replace terramycin or aureomycin. For example, when tested against *M. pyogenes var. aureus* (H) bacitracin and terramycin were synergistic(7) but neither erythromycin nor carbomycin showed this effect when combined with bacitracin. Using another strain of *M. pyogenes var. aureus* (Sh), it was shown that erythromycin and bacitracin were synergistic over a wide range of concentrations including bactericidal doses of bacitracin. Against a third strain of this organism (Da), bacteriostatic doses of bacitracin were additive when combined with the new drugs (Table I). As with other Group II antibiotics, the behavior of erythromycin and carbomycin in combination with Group I drugs was unpredictable and seemed to depend, at least in part, upon the sensitivity of the test bacteria to the Group I drug. The available evidence therefore placed erythromycin and carbomycin into Group II of the above scheme. A paper by Rantz and Randall(12) published while this work was in progress likewise places erythromycin into Group II. Experiments are in progress to confirm this *in vitro* classification by results of treatment of experimental infections.

There are two principal reasons for the clinical simultaneous use of 2 antimicrobial drugs in treating an infection due to a single microbial species(6,13): a) One antimicrobial drug delays the emergence of variants resistant to the other drug. b) The bactericidal rate or bacteriostatic effect of the drug mixture is far greater than that of either drug alone in comparable concentration. It had already been shown that the addition of Group I drugs to erythromycin or carbomycin delays the emergence of resistant variants which tend to shorten greatly the useful length of drug administration(5). The work presented here classifies erythromycin and carbomycin with other Group II agents and indicates that on occasions striking synergism with Group I drugs may be encountered. Using a survey technic to be reported later, both erythromycin and carbomycin showed some form of positive summation of action in combination with Group I drugs against 11 strains of recently isolated *M. pyogenes*. The antagonism between these agents and Group I antibiotics, as with other Group II drugs, is probably of limited clinical significance for reasons described elsewhere(6). Therefore, it appears that use of erythromycin and carbomycin in combination with other antimicrobial agents deserves consideration.

Summary. With respect to their behavior in combinations with other antibiotics, as measured by bactericidal rate *in vitro*, erythromycin and carbomycin fall in a group with aureomycin, chloramphenicol and terramycin. The classification of these agents in a tentative scheme of combined antibiotic action is discussed in terms of laboratory tests and clinical application.

1. McGuire, J. M., Bunch, R. L., Anderson, R. C., Boaz, H. E., Flynn, E. H., Powell, H. M., and Smith, J. W., *Antibiot. and Chemother.*, 1952, v2, 281.

2. Tanner, F. W., Jr., English, A. R., Lees, T. M., and Routien, J. B., *Antibiot. and Chemother.*, 1952, v2, 441.

3. Haight, T. H., and Finland, M., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 175.

4. Finland, M., Wilcox, C., Wright, S. S., and Purcell, E. M., PROC. SOC. EXP. BIOL. AND MED., 1953, v81, 725.

5. Purcell, E. M., Wright, S. S., and Finland, M., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 124.

6. Jawetz, E., *Arch. Int. Med.*, 1952, v90, 301.

7. Jawetz, E., Gunnison, J. B., Bruff, J. A., and Coleman, V. R., *J. Bact.*, 1952, v64, 29.

8. Gunnison, J. B., Shevky, M. C., Bruff, J. A., Coleman, V. R., and Jawetz, E., *J. Bact.*, in press.

9. Jawetz, E., and Gunnison, J. B., *Antibiot. and Chemother.*, 1952, v2, 243.

10. Gunnison, J. B., Jawetz, E., and Coleman, V. R., *J. Lab. and Clin. Med.*, 1950, v36, 900.

11. Gunnison, J. B., Coleman, V. R., and Jawetz, E., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 549.

12. Rantz, L. A., and Randall, E., *Antibiot. and Chemother.*, 1950, v2, 645.

13. Dowling, H. F., Lepper, M. H., and Jackson, G. G., *J.A.M.A.*, 1953, v151, 813.

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