# Cross Resistance to Antibiotics: Effect of Exposures of Bacteria to Carbomycin or Erythromycin *in Vitro*.* (20000)

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Among the antibiotics currently in wide use, cross resistance to one antibiotic resulting from repeated or prolonged exposures to another has been demonstrated to occur between aureomycin and terramycin(1-5), between either of these agents and chloramphenicol (1-4), and between streptomycin and neomycin(3,6). In the course of studies originally designed to investigate some bacteriological aspects of the new antibiotic carbomycin (Magnamycin)(7) and its clinical potentialities, it became apparent at once that the antibacterial spectrum of this new agent closely parallels that of erythromycin, both qualitatively and quantitatively. A study of cross resistance between these 2 agents was, therefore, undertaken. In this paper are reported the results of parallel tests for sensitivity of a number of bacterial strains to carbomycin and erythromycin together with the results of tests for resistance and cross resistance to these agents resulting from repeated subcultures of bacteria in increasing concentrations of each of these antibiotics.

*Materials and methods.* Both the erythromycin and the carbomycin used in this study were obtained in the form of the purified crystalline base; the labeled potency was 890 μg/mg of erythromycin and 830 μg/mg of carbomycin. The methods used were similar to those employed in similar studies previously reported from this laboratory(3,6,8). The tests for sensitivity were done by an agar-plate dilution method (10% horse blood was incorporated in the agar throughout this study) and the attempts to increase resistance were made by serial subcultures of each strain from the agar plate containing the maximum concentration of antibiotic on which good growth occurred in the sensitivity test to another series of agar plates containing similar 2-fold serial dilutions of the antibiotic. For some of the cross resistance tests with carbomycin, a number of the strains used had been developed in other studies(8) by subcultures on graded concentrations of penicillin, streptomycin or erythromycin and also with combinations of penicillin and erythromycin(8) and of streptomycin and erythromycin (8). In a number of the latter studies, some of the serial subcultures had been made daily, and broth was used instead of agar for the subcultures of some of the strains(8); in the present study the subcultures on carbomycin agar were made after 48 hours of incubation in each instance. In this study the sensitivity of a strain to erythromycin is expressed as the minimum concentration of antibiotic in μg of the active base per ml of media which completely inhibits visible growth in 24 hours (M.I.C.); in the case of carbomycin, M.I.C. is expressed in terms of actual weight per ml.

*Results. Sensitivity of bacteria to carbomycin and erythromycin.* Parallel tests for sensitivity to carbomycin and erythromycin were carried out on 74 strains of a large variety of bacterial species. Some of these organisms were stock laboratory strains, but the great majority were freshly isolated from patients with acute infections. The results are listed in Table I. It is seen that the gram-positive organisms and the gram-negative cocci were all highly sensitive and the strains of *Hemophilus* were moderately sensitive while the coliform and the enteric bacilli were all resistant to both carbomycin and erythromycin. Of particular interest, however, is the fact that, with the exception of 3 strains which were equally sensitive to both agents, all of the sensitive strains required greater concentrations of carbomycin than erythromycin to

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produce the same degree of inhibition; of the 55 sensitive strains, 45 required 4 or 8 times as much carbomycin as erythromycin; 5 required 16 times as much and 2 required twice as much carbomycin as erythromycin for complete inhibition.

TABLE I. Sensitivity of 74 Bacterial Strains to Carbomycin and Erythromycin.*

| Organism | | | M.I.C.,† μg/ml Carbomycin | M.I.C.,† μg/ml Erythromycin |
|---|---|---|---|---|
| Pneumococcus, type | 5 | | .2 | .05 |
| | 7 | | .4 | .05 |
| | 10 | | .4 | .05 |
| | 17 | | .4 | .05 |
| | 18 | | .4 | .05 |
| | 18 | | .4 | .05 |
| | 19 | | .1 | .02 |
| | 4 | | .4 | .05 |
| | 18 | | .1 | .02 |
| | 5 | | .1 | .02 |
| | 10A | | .2 | .02 |
| | 19 | | .8 | .1 |
| *Streptococcus C203, | Group | A | .2 | .05 |
| * 98 | | A | .1 | .02 |
| | | A | .8 | .05 |
| | | B | .8 | .2 |
| * C3 | | D | .8 | .2 |
| * D76 | | D | .8 | .8 |
| | | D | 6.3 | 6.3 |
| | | D | .4 | .05 |
| | | D | 1.6 | .4 |
| | | D | 1.6 | .4 |
| * K131 | | E | .8 | .05 |
| * 166B | | G | .4 | .05 |
| * 167B | | G | .4 | .05 |
| Microaerophilic streptococcus | | | .8 | .1 |
| | | | .4 | .1 |
| *Streptococcus mitis* | | | .2 | .05 |
| | | | .4 | .05 |
| | | | .4 | .05 |
| | | | .4 | .1 |
| | | | .4 | .05 |
| | | | .4 | .05 |
| | | | .8 | .05 |
| | | | .4 | .05 |
| | | | .4 | .1 |
| | | | .4 | .1 |
| *Staphylococcus albus* | | | 6.2 | 1.6 |
| *aureus* | | | 1.6 | .4 |
| | | | 3.1 | .4 |
| | | | 1.6 | .4 |
| | | | 1.6 | .4 |
| **Sarcina lutea* | | | .1 | .02 |
| **Bacillus cereus* No. 5 | | | 1.6 | .4 |
| Diphtheroid | | | .8 | .05 |
| | | | " | " |
| *Neisseria meningitidis*, 2A | | | .2 | .1 |
| | | | .4 | .4 |
| | | | .8 | .4 |
| *gonorrhoea* | | | 1.6 | .4 |
| | | | " | " |
| *Haemophilus influenzae* | | | 12.5 | 3.1 |
| | | | 50 | 12.5 |
| | | | 50 | 12.5 |
| | | | 6.3 | .8 |
| *Escherichia coli* | | | 200 | 100 |
| " | " | | | 100 |
| " | " | | | >200 |
| *Aerobacter aerogenes* | | | >200 | 200 |
| " | " | | | |
| " | " | | | >200 |
| *Klebsiella pneumoniae*, | | A | | |
| " | " | A | 200 | 200 |
| " | " | A | | |
| " | " | B | | |
| *Pseudomonas aeruginosa* | | | | |
| " | " | | | |
| " | " | | | |
| " | " | | >200 | >200 |
| " | " | | | |
| *Salmonella salinatis* | | | | |
| " | *manhattan* | | | |
| " | *st. paul* | | | |
| " | *oranienberg* | | | |

* Indicates stock strains; the numbered strains of groups D, E and G streptococci were obtained from Dr. Rebecca Lancefield; all other strains were freshly isolated from patients in the hospital by Miss Marion E. Lamb.

† Minimum complete inhibiting concentration.

*Cross resistance between carbomycin and erythromycin.* Five of the strains which had been made resistant to erythromycin in the previous study of Haight and Finland(9), together with the corresponding parent strains which had been transferred in parallel on antibiotic-free agar were tested simultaneously for their resistance to carbomycin and erythromycin. The same 5 parent strains were also subjected to a second series of parallel subcultures on antibiotic-free agar and on agar containing increasing concentrations of carbomycin; after 20 such subcultures, all of the strains of the second series were likewise tested at the same time for their sensitivity to carbomycin and erythromycin. The results are shown in Table II, and the progressive increases in resistance to carbomycin resulting from the repeated transfers in the presence of the homologous antibiotic are shown in Fig. 1. Marked increases in resistance to carbomycin developed in each of the strains which had been repeatedly subcultured in the presence of that antibiotic. The strains which had been repeatedly exposed to erythromycin, with the exception of Strep. C203, increased simi-

TABLE II. Cross Resistance between Carbomycin and Erythromycin.

| Strain | | M.I.C.,* μg/ml Carbomycin | M.I.C.,* μg/ml Erythromycin |
|---|---|---|---|
| Series 1† | | | |
| Staphylococcus 192 | —0 ‡ | 3.1 | .4 |
| | ER | >800 | >800 |
| 193 | —0 | 1.6 | .4 |
| | ER | >800 | >800 |
| Enterococcus | —0 | 3.1 | .4 |
| | ER | 200 | >800 |
| *Streptococcus mitis* | —0 | .1 | .05 |
| | ER | 1.6 | .4 |
| C203 | —0 | .2 | .05 |
| | ER | .4 | .1 |
| Present series (Fig. 1) | | | |
| Staphylococcus 192 | —0 | 3.1 | 1.6 |
| | CR | 400 | 100 |
| 193 | —0 | 3.1 | 1.6 |
| | CR | >800 | 100 |
| Enterococcus | —0 | 3.1 | .2 |
| | CR | 50 | 3.1 |
| *Streptococcus mitis* | —0 | .2 | .05 |
| | CR | 25 | .2 |
| C203 | —0 | .2 | .05 |
| | CR | 6.3 | .8 |

* Minimum complete inhibiting concentration.
† See Haight & Finland(9), Fig. 1.
‡ 0 = repeated subcultures on antibiotic-free media; ER = after repeated transfers on erythromycin; CR = after repeated subcultures on carbomycin.

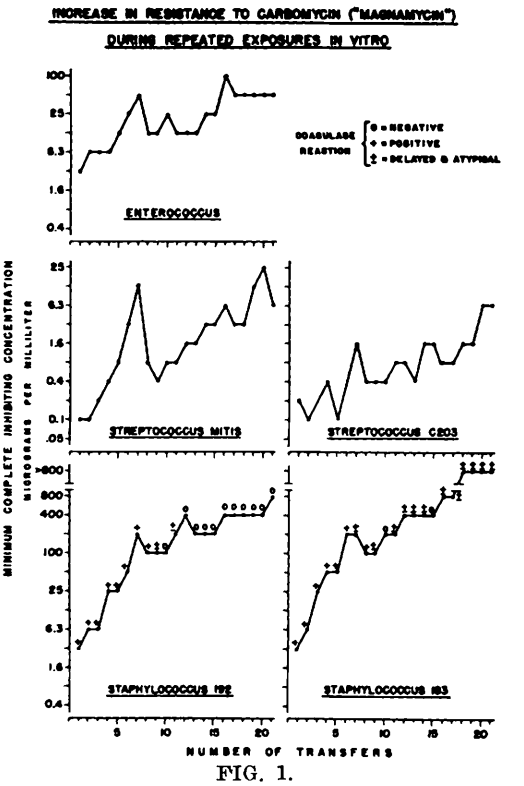

FIG. 1.

larly in resistance to the homologous agent. Cross resistance between the carbomycin and the erythromycin was complete in every instance. Moreover, the ratio of the minimum complete inhibiting concentration of carbomycin to that of erythromycin for each of the parent strains and for their resistant variants was essentially similar to that observed in Table I, except in the case of the carbomycin-resistant strain of *Streptococcus mitis* which required 128 times as much carbomycin as erythromycin. *Additional tests* for sensitivity to carbomycin and erythromycin were carried out simultaneously with 7 strains of staphylococci and the variants of these strains derived by serial subcultures in the presence of erythromycin, streptomycin or both in combination(8); similar tests were also done with 5 additional strains of staphylococci and their respective variants derived by serial transfers on erythromycin, streptomycin, or a combination of both. The results are given in Table III. Each of the parent strains and their variants required 4 to 16 times as much carbomycin as erythromycin to produce complete inhibition. Increases in resistance to erythromycin was accompanied, in every instance, by a parallel increase in resistance to carbomycin.

*Effect of development of carbomycin resistance on coagulase production by staphylococci.* The results of coagulase tests carried out daily during the successive transfers of 2 staphylococcal strains in increasing concentrations of carbomycin are shown in Fig. 1. *Staph. 192* lost its coagulase activity between the 9th and 12th transfer in the presence of carbomycin; in the case of *Staph. 193*, on the other hand, the coagulase activity became altered at about the same stage of the transfers and, except on 2 occasions, this activity was reduced and delayed in appearance but still demonstrable. In previous studies, coagulase tests done during or after successive exposures of other strains of staphylococci to erythromycin yielded irregular results. In the studies of Haight and Finland(9) on 4 strains, including the 2 just mentioned which are shown

TABLE III. Cross Resistance between Erythromycin and Carbomycin.*

A. Effect of repeated exposures to penicillin and/or erythromycin

| Strain | Min complete inhibiting conc., μg/ml: Erythromycin 0 | P—R | E—R | P+E—R | Carbomycin 0 | P—R | E—R | P+E—R |
|---|---|---|---|---|---|---|---|---|
| Staphylococcus 1 | | | .4 | .2 | | 3.1 | 3.1 | 3.1 |
| 2 | | | 25 | .8 | | 3.1 | 100 | 25 |
| 3 | | | 6.3 | 3.1 | | 3.1 | 100 | 50 |
| 4 | .4 | .4 | 800 | 3.1 | 6.3 | 6.3 | 100 | 25 |
| 5 | | | 200 | 1.6 | | 6.3 | >400 | 12.5 |
| 6 | | | 100 | >800 | | 6.3 | >400 | >400 |
| 7 | | | 6.3 | .8 | | 12.5 | 100 | 6.3 |

B. Effect of repeated exposures to streptomycin and/or erythromycin

| | 0 | S—R | E—R | S+E—R | 0 | S—R | E—R | S+E—R |
|---|---|---|---|---|---|---|---|---|
| Staphylococcus S | | | 12.5 | 6.3 | 3.1 | | 50 | 25 |
| W | | | 12.5 | .8 | 3.1 | | 50 | 3.1 |
| M | .4 | .4 | 6.3 | 6.3 | 3.1 | 3.1 | 25 | 25 |
| V | | | >100 | 6.3 | 6.3 | | >100 | 25 |
| A | | | 12.5 | 1.6 | 3.1 | | 50 | 6.3 |

* Strains used in study of Purcell, Wright and Finland(8).

0 = no previous exposure to antibiotics; R = previous transfers with antibiotics; P = penicillin; S = streptomycin; E = erythromycin.

in Fig. 1, the coagulase producing activity in the case of each strain first became poor and then was completely lost during the successive subcultures with erythromycin. In subsequent studies(8), other strains of staphylococci which increased in resistance during repeated transfers in the presence of erythromycin generally retained their coagulase activity apparently unaltered. Other distinctive properties of the staphylococci were not demonstrably altered during the course of their repeated exposures to carbomycin. The basis of these differences in effects on coagulase activity is not clear.

*Discussion.* The data presented in this paper indicate a very close relationship between carbomycin and erythromycin with respect to their antibacterial activity; this relationship is both qualitative and quantitative. It appears from these data that, weight for weight, erythromycin is from 4 to 16 times as active as carbomycin against almost all of the bacterial strains for which the sensitivity to these agents has been compared. High grades of resistance to carbomycin and erythromycin develop with considerable ease and rapidity during the course of repeated subcultures of susceptible staphylococci and of certain streptococci in the presence of each of these antibiotics. Moreover, increases in resistance resulting from exposures to the homologous antibiotic are accompanied by increases in resistance of about the same order of magnitude to the other agent to which the organisms had not previously been exposed. These findings and the claims that each of these antibiotics are active against rickettsias and against the psittacosis and related viruses (7,10) suggest the possibility that the 2 agents may be chemically very closely related in spite of the apparently divergent physical constants presented in the original papers describing the discovery of erythromycin(10) and carbomycin(7); the data, however, do not constitute valid proof of such a chemical relationship. The comparisons of the biological activities of these 2 substances are reminiscent of the comparative activities of aureomycin and terramycin, these antibiotics have many distinctive physical characteristics but they have recently been shown to have very similar basic chemical structures(11,12).

*Summary and conclusions.* The antibacterial spectrum of carbomycin parallels very closely that of erythromycin. Both of these new antibiotics are highly active against gram-positive and gram-negative cocci, moderately active against strains of *Haemophilus* and are essentially inactive against coliform and enteric bacilli. Against susceptible bacterial strains erythromycin is usually from 4 to 16 times more active than carbomycin, weight for

weight. Repeated subcultures of staphylococci and of certain strains of streptococci in the presence of increasing concentrations of either one of these antibiotics result in fairly rapid and marked increases in resistance not only to the antibiotic to which it was exposed but to the other agent as well.

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## Role of the Pancreas in Prevention of Fatty Liver in the Hypophysectomized-Thyroidectomized Dog.* (20001)

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The pancreas, by virtue of its exocrine function, exerts a profound influence on the liver. This has been clearly brought out in the case of the fatty liver that appears in the completely depancreatized dog receiving insulin(1). It is shown in this report that the digestive function of the pancreas may be implicated in the development of another type of fatty liver, namely, that observed in the hypophysectomized - thyroidectomized (HT) dog. This type of fatty liver is prevented if choline in the free form is added to the diet (2). Evidence presented here shows that extra methionine is also efficacious. That the fatty liver in the doubly-operated dog is the result of pancreatic insufficiency is indicated by the additional finding that its development can be prevented by feeding raw pancreas.

*Experimental.* Nine dogs were used in this study. Throughout their stay in the laboratory they were fed, daily, 30 g of lean meat per kg of body weight, in addition to sucrose, vitamins, and a salt mixture. The exact composition of this diet has been described elsewhere(3). The pituitary glands were first removed, and one to 2 weeks later the thyroids were excised. The animals were then divided into 3 groups (Table I). The control dogs (Table I) were fed just the lean meat diet described above. The second group was fed, in addition, 25 g of pancreas with each meal. The third group was fed 2 g of methionine with each meal. To ensure the exact dietary intakes recorded for each dog, forced feeding was resorted to at times. All of the dogs were maintained for not less than 749 days. This period exceeds the minimum time necessary for the consistent development of the fatty liver in HT dogs(2). At the end of the period of observation, livers and blood were removed, and their lipide contents determined by methods previously described(4).

*Results.* In a preceding paper it was shown that hypophysectomy and thyroidectomy result in the development of a fatty liver in the dog in 217 to 419 days(2). In the present study the livers of the 2 control dogs contained 21.6% and 27.4% total fatty acids (Table I). From 2 to 3% total fatty acids were found in the livers of the dogs fed pan-

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