Drug Antagonism between Lincomycin and Erythromycin

Abstract. *An antagonistic action can be demonstrated between lincomycin, a new antibiotic, and erythromycin, when the two drugs are allowed to diffuse into the same area of an agar plate seeded with a strain of* Staphylococcus *which is resistant to erythromycin but sensitive to lincomycin. The increase in the minimum inhibitory concentrations of lincomycin in the presence of erythromycin may be significant in clinical application. The antagonism does not depend on a reaction between the two antibiotics, but appears to be the result of an altered metabolism stimulated by erythromycin on erythromycin-resistant staphylococci.*

The discovery of lincomycin, a new antibiotic produced by *Streptomyces lincolnensis* var. *lincolnensis* sp. n., was reported by Mason *et al.* in 1962 (*1*). Lincomycin is chemically distinct from all other antibiotics now available. It is isolated as a white crystalline solid and is stable in the dry state and in aqueous solutions. Although lincomycin does not possess the characteristic macrocyclic lactone ring in its structure, for convenience it has been placed in the macrolide group because of its similar antibacterial spectrum (*2*). With the exception of *Streptococcus faecalis*, lincomycin is highly effective against most Gram-positive cocci. Lincomycin is not cross-resistant with penicillin, novobiocin, tetracycline, chloromycetin, streptomycin, or neomycin, but is partially cross-resistant with the erythromycin group (*3*).

During a study of the changes in the drug sensitivity of staphylococci, we observed a distinct antagonistic reaction between erythromycin and lincomycin (Fig. 1). To demonstrate this action, it is necessary to use a strain of *Staphylococcus* which is resistant to erythromycin but sensitive to lincomycin. The bacteriostatic action of lincomycin is inhibited if both drugs, lincomycin and erythromycin, are allowed to diffuse into the same area so that a mixing occurs in the agar plate. In the area of mixing, antibiotic antagonism is evident.

The antagonistic effect of erythromycin on lincomycin was titrated by two different methods. We used first the Oxford cylinder-plate-assay method, which depends on the diffusion of antibiotics from a steel cylinder into the inoculated agar medium. The minimum inhibitory concentrations for the strains of *Staphylococcus aureus* used were just slightly in excess of 1 μg of lincomycin per milliliter. The antagonistic action of erythromycin and lincomycin was demonstrated by mixing 1, 2, 4, 6, 8, and 10 μg/ml of erythromycin with a medium containing 100 μg/ml of lincomycin. The mixtures were incubated for 2 hours at 37°C, after which 0.1 ml was transferred to Oxford cylinders, where an erythromycin-resistant, lincomycin-sensitive strain of *S. aureus* was used as an indicator organism. The results of this test indicated that 0.6 μg/ml of erythromycin inhibited the action of approximately 10.0 μg/ml of lincomycin.

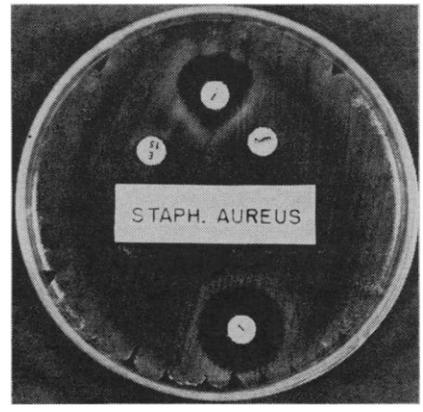

Fig. 1. Erythromycin-lincomycin antagonism demonstrated by the decrease of zone of inhibition. Erythromycin discs (*E*) contain 2 and 15 units, discs labeled "*1*" contain 2 μg of lincomycin.

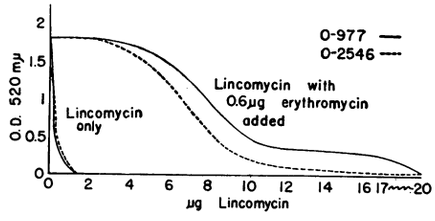

Fig. 2. Changes in the minimum inhibitory concentration of lincomycin caused by the addition of erythromycin.

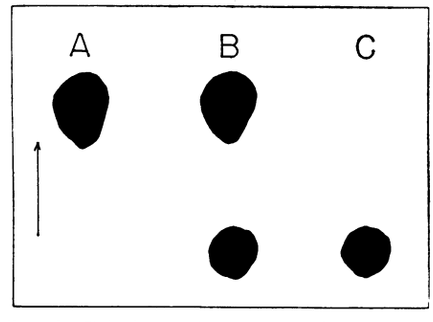

Fig. 3. Thin-layer chromatographic separation of lincomycin and erythromycin. Superimposed indicator layer consisting of a 3-mm layer of blood agar seeded with a hemolysis-producing strain of *Staphylococcus* which is sensitive to both drugs. *A*, Lincomycin; *B*, combination; *C*, erythromycin.

A turbidimetric assay was used as a second method. Two series of dilutions of lincomycin, ranging from 1 to 20 μg/ml in trypticase soy broth, were employed. Each of the test tubes in one series received an inoculum of 0.05 ml of an 18-hour broth culture of the *S. aureus* indicator and was incubated for 24 hours. Each tube in the second series received, in addition to the above inoculum, 0.6 μg of erythromycin per milliliter and was incubated for 24 hours. The tube with the lowest concentration of lincomycin which inhibited the growth of the organism was taken to be the minimum inhibitory concentration (MIC) of lincomycin. An increase in the MIC was produced by the addition of erythromycin (Fig. 2).

The nature of the antagonism between these two antibiotics is not known. Thin-layer chromatography showed that the phenomenon was not due to simple chemical combination. Pure erythromycin and lincomycin were applied individually to the chromatograph, as was a mixture of the two which had been allowed to stand for 4 hours so that any possible chemical combination might occur. Figure 3 indicates that neither chemical combination nor deterioration of antibiotic content took place. Further, the antagonism was not due to the resistant organism producing a substance, in the presence of erythromycin, which inactivated the lincomycin. Bioassays on the supernatant from the medium containing both antibiotics, in which the resistant organism had grown, showed no changes in the original antibiotic concentrations.

Although reports indicating that this kind of antagonism may become clinically important have been rare (*4*), this does not imply that antibiotic antagonism is unimportant. Because of multiple drug therapy, the manifestations of the phenomenon are masked.

Our experiments in vitro indicate that antagonism would occur between lincomycin and erythromycin at the concentrations reached by these drugs in the serum as a result of ordinary therapy. The antagonism may thus be of clinical significance when an eryth-

romycin-resistant *Staphylococcus* is being treated. Since no evidence of a physical or chemical reaction between the two drugs was demonstrated, it seems possible that with erythromycin-resistant staphylococci, erythromycin stimulates a metabolic pathway which circumvents the action of lincomycin on the individual cell.

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Probiotics: Growth-Promoting Factors Produced by Microorganisms

Abstract. *Several species of protozoa, during their logarithmic phases of growth, produce substances that prolong the logarithmic phase in other species. The effect is not as striking as the inhibition of growth caused by antibiotics, but a consistent 50-percent increase in growth has been obtained with* Tetrahymena pyriformis *in response to a factor produced by* Colpidium campylum.

Certain kinds of microorganisms growing in a culture medium often produce antibiotics inhibitory to the growth of other organisms. There have been several reports, however, of an opposite effect, growth stimulation, when the culture medium has been conditioned by a previous inoculation. The name given by Robertson (*1*) in the early 1920's to the effect observed in the growth of *Enchelys* and *Colpoda* was allelocatalysis. Several other workers (*2*) failed to confirm this allelocatalytic effect, but at that time there was little appreciation of the importance of axenic cultures and the desirability of chemically defined media for such experiments.

Before the development of a chemically defined medium for the ciliate now known as *Tetrahymena pyriformis*, Kidder (*3*) demonstrated that crude peptone medium could be conditioned by *Tetrahymena* so that organisms subsequently introduced into it grew more rapidly and to a greater concentration than in control cultures. Under certain conditions, inhibitory effects were also observed. In our laboratory, growth-promoting effects were obtained with different kinds of crude media and with different species of ciliates (*4*). Substances produced by *Tetrahymena* favored the growth of *Stylonychia*. Products of *Stylonychia* increased the growth of *Paramecium*, while conversely, the products of *Paramecium* favored the growth of *Stylonychia*. In all these cases, the factor responsible for the increased growth was nondialyzable and was thermolabile. In the case of *Colpidium campylum*, however, the product was not as readily destroyed by heat and yet it had a significant growth-promoting effect on *Paramecium caudatum* (*5*). It was also found possible to grow *Colpidium* in a chemically defined medium so that the unknown products, which we designate "probiotics," could be readily separated from the known components of the culture medium (*6*). The results obtained in this laboratory with ciliates are reminiscent of some of the reports in the bacteriological literature of growth-promoting effects of certain peptides. These were first described under the designation "strepogenin" (*7*). More recent work has confirmed the fact that several different kinds of peptides have growth-promoting activity for several different species of bacteria (*8*).

To test the growth-promoting activity of the protozoan product, we used two rapidly growing species that could be cultivated in a chemically defined medium. *Colpidium campylum* was used to produce the thermostable probiotic and *Tetrahymena pyriformis* (strain W) was used as the assay organism. The medium was a modification of medium A originally developed by Kidder and Dewey (*9*) for *Tetrahymena*. This had subsequently been changed to allow growth of *Colpidium campylum* (*10*), and another improvement by Holz *et al.* (*11*) permitted the growth of *Glaucoma chattoni* A without protein in the medium. To obtain satisfactory growth of both *Colpidium* and *Tetrahymena* in the same medium, it was necessary to make additional changes in the composition of the medium by inclusion of fatty acids and a sterol (Table 1). Growth of both organisms in this medium was near the maximum reported by other investigators; growth was evaluated by

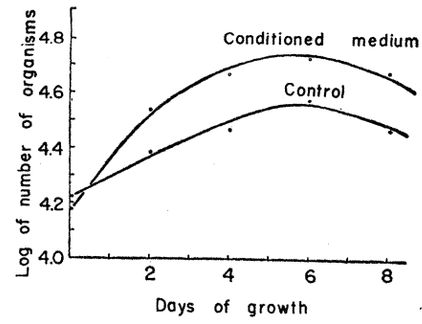

Fig. 1. Stimulation of *Tetrahymena pyriformis* by the probiotic from *Colpidium campylum*.

taking the average of four counts at each reading (*12*). The probiotic factor produced by *Colpidium* consistently prolonged the logarithmic growth phase of *Tetrahymena* by as much as 50 percent (Fig. 1), and there was a slight increase in the rate of growth in most of the experiments. Apparently, the main effect on growth regulation consisted in delaying the transition to the stationary phase and maintaining this plateau longer than in control cultures.

The probiotic effect was lost when the conditioned medium was subjected

Table 1. The chemically defined medium in which *Colpidium campylum* and *Tetrahymena pyriformis* were grown. Quantities expressed as micrograms per milliliter of final medium. Final *p*H adjusted to 7.0 with 0.1*N* NaOH. The stigmasterol and the vitamins were autoclaved separately and added aseptically to the other components which were mixed and sterilized together. The glucose was Seitz-filtered and added aseptically to the medium.

Substance	Quantity	Substance	Quantity
L-Alanine	25	Oleic acid	1.25
L-Arginine	100		
L-Aspartic acid	50	Stigmasterol	2
Glycine	25		
L-Glutamic acid	75	Calcium pantothenate	2
L-Histidine	50	Nicotinamide	4
L-Isoleucine	150	Pyridoxal HCl	4
L-Leucine	150	Riboflavin	2
L-Lysine HCl	125	Folic acid	1
L-Methionine	150	Thiamine HCl	6
L-Phenylalanine	75	Biotin	0.004
L-Proline	50	DL-6-Thioctic acid*	0.004
L-Serine	200		
L-Threonine	150		
L-Tyrosine	50	Sodium ethylenediamine tetraacetate	20
L-Tryptophan	50		
L-Valine	75		
Guanylic acid	75	$MgSo_4 \cdot 7H_2O$	40
Adenylic acid	30	$CaCl_2 \cdot 2H_2O$	20
Cytidylic acid	75	$(NH_4)_2SO_4$	10
Uridylic acid	20	$CuCl_2 \cdot 2H_2O$	2
Sodium acetate	570	$FeCl_3 \cdot 6H_2O$	0.5
		$MnCl_2 \cdot 4H_2O$	0.1
Glucose	2500	$ZnCl_2$	0.02
Linoleic acid	3.75	KH_2PO_4	570
		K_2HPO_4	570

* α-Lipoic acid.