SYNERGY BETWEEN FOSFOMYCIN AND ARENAEMYCIN

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Synergy was evident in zones of inhibition between neighboring filter discs containing fosfomycin and an unidentified fermentation broth placed on the surface of agar growth medium seeded with *Bacillus megaterium* (S. MOCHALES; personal communication). The antibiotic was isolated and identified as arenaemycin (T. MILLER and K. WILSON; personal communication). We report in this communication that arenaemycin and fosfomycin indeed are synergistic *in vitro*.

The skewed inhibition zones between filter discs on seeded plates indicates that pure arenaemycin and fosfomycin are synergistic (Fig. 1). Quantitative synergy was measured by determining the MICs of fosfomycin and arenaemycin alone and in combined concentrations below the MICs. The data are summarized in Table 1.

Arenaemycin E is reported to be a selective and strong inhibitor of glycerolaldehyde-3-phosphate dehydrogenase[1]. A proposed site of action of arenaemycin C is on pyruvate kinase[2]. Fosfomycin is an inhibitor of phosphoenolpyruvate: UDP-GlcNac-3-enolpyruvyl transferase and has little or no activity on other phosphoenolpyruvate utilizing enzymes[3]. Whether inhibition of these enzymes by arenaemycins and fosfomycin could explain the synergy is not clear. Both fosfomycin[4] and arenaemycin (E. O. STAPLEY and T. W. MILLER; personal communication) induce spheroplast formation in sensitive Gram-negative bacteria. Thus, they do inhibit steps in cell wall synthesis and the FICI shows definite synergy.

Arenaemycins, also called pentalenolactones, have been reported in fermentation broths from several species of *Streptomyces*[5]. Antibiotic PA132[6] and AA-57[7] belong to this group of antibiotics. Members of this group have not been absent from some of the antibiotic screening

Fig. 1. Synergy between fosfomycin and arenaemycin.

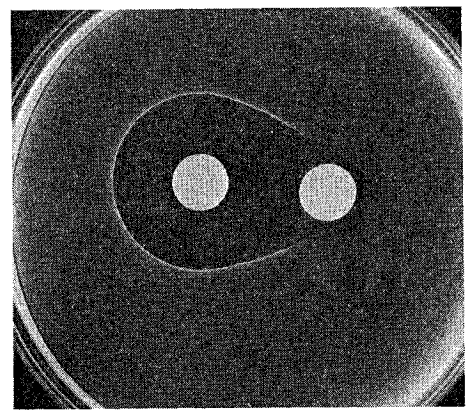

Plate containing 5 ml of nutrient agar seeded with *Proteus vulgaris* MB-838 1% was incubated at 37°C for 18 hours. Filter disc on left contains fosfomycin 0.25 μg and the disc on right contains 1.0 μg of arenaemycin.

Table 1. *In vitro* antibacterial activity of fosfomycin and arenaemycin.

Test organism	MIC (μg/ml)		FICI
	Fosfomycin	Arenaemycin	
Proteus vulgaris MB-838	0.1	1.0	0.2
Salmonella gallinarum MB-1287	0.25	0.05	0.4

Test organisms were grown 20 hours at 37°C with agitation in nutrient broth containing 100 μg/ml of glucose-6-phosphate. 0.1 ml of growth was added to tubes containing 9.9 ml of melted cooled nutrient agar supplemented with 100 μg/ml of glucose-6-phosphate and containing fosfomycin and arenaemycin alone and in various combinations. Contents of the tubes were vortexed and poured into petri plates. The MICs were determined after 20 hours incubation at 37°C. The FICI[10] is the sum of the fractional inhibitory concentrations. The fractional inhibitory concentration of each antibiotic is the amount in combination that produces the MIC expressed as a fraction of the MIC of the antibiotic alone. We accept an FICI of ≤ 1.0 as showing synergy.

programs in our institution (E. O. STAPLEY and T. W. MILLER; personal communication).

The biosynthesis of arenaemycin/pentalenolactone antibiotics has received some attention (see citations 5 and 8 for references). Some members of the group have been reported to have antiviral activity[9].

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