Sargent, M. L. Effects of four antibiotics on growth and periodicity of a rhythmic strain of Neurospora. The effects of actinomycin D, cycloheximide, mitomycin D, and puromycin on the circadian conidiation rhythm and linear grwth rate of the bd (brand) strain of Neurospora (Sargent and Woodward 1969 J. Bacteriol. 97: 861) hove been studied. There inhibitors affect

circadian rhythms in certain algal species, so we wished to ascertain their usefulness for the study of Neurospora rhythms.

A <u>bd</u> strain (MLS E7a) was grown on Vogel's salts, 0.3% glucose, 0.5% arginine-HCl, and 1.5% agar in growth-tube cultures under conditions of continuous darkness and constant temperature (25°C) and relative humidity (70%). The octinomycin D was supplied through the courtesy of Merck Sharpe and Dohme, Rahway, New Jersey, while the cycloheximide, mitomycin C and puromycin-diHCl were abtained from Sigma Chemical Co., St. Louis, Missouri. The filter-sterilized antibiotics were added to worm medium (45-50°C) just prior to dispensing it into growth tubes.

None of the antibiotics were able to alter significantly the period of the conidiation rhythm; therefore, the basic oscillator causing rhythmicity is not affected by the inhibitors at the concentrations used. Mitomycin C, up to concentrations of 108 μ g/ml, has no significant effect on either growth rate or conidiation.

Figure 1 illustrates the effect of three antibiotics on linear growth rate. It is evident that (1) cycloheximide is on effective inhibitor at very low concentrations, (2) high concentrations of puromycin are needed to inhibit growth, and (3) actinomycin D is effective at internediate concentrations. The effect of octinomycin D is unique in that it is cumulative; i.e., grwth is normal for the first 2-3 days of an experiment, but subsequently growth becomes progressively slower. The growth-rate curve for actinomycin D (Figure 1) is based on data collected on the eighth day after inoculation. Rhythmicity is obscured by the inhibition of conidiation by cycloheximide (above 0.050 μ g/ml) and actinomycin D (above 1.0 μ g/ml). Puromycin has no effect on conidiation even at the highest concentration used.

It should be noted that the <u>bd</u> strain tested conidiates well in growth-tube cultures, and has a linear growth rate (about 40 mm/day) approximately one-half that of the

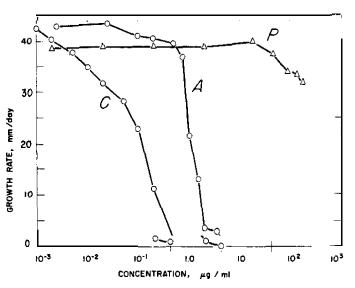


Figure 1. Effect of actinomycin D (A), cycloheximide (C), and puromycin (P) on the linear growth rate of a \underline{bd} strain in growth-tube cultures.

parent wild type strain, although the bd strain is nutritionally wild type. In liquid cultures the dry-weight grwth rate of bd strains is like that of wild type. It is conceivable that these four antibiotics would have different ranger of effectiveness on the growth of wild type strains. I thank A.S.Sussman for advice and support during the course of this work. -- Department of Botany, University of Illinois, Urbana, Illinois 61801.