Effects of cycloheximide at low concentrations

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Abstract
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presence of inhibiting concentrations of thorazine were characterized by a “rice-like” morphology. This peculiar morphology persisted throughout the growth of the culture, even after inhibition had been overcome. No growth occurred over a period of 5 days in cultures containing thorazine in excess of 2 \times 10^{-4} \text{ M}.

Antimycin A: Inclusion of 1 \mu g/\text{ml.} of antimycin A in the culture medium has been observed to lengthen the moss doubling time of the mycelium during the exponential phase of growth from 3 to 7 hours.


2. Hyphal tip of microcultures (Wilson and Garnjobst 1966 Genetics 53:621) develop abnormal morphology after exposure to concentrations of 0.6, 0.7, or 0.8 \mu g/\text{ml}. This change is accompanied by pronounced cytoplasmic flow into the tips, with consequent swelling and dichotomous branching even in the presence of a hypertonic (14\%) sucrose solution.

3. Regeneration into punctured cells, typically 100\% within 45 minutes at 30\%, is totally inhibited in the presence of 1.0 \mu g/\text{ml.} Of a group of cells individually injected with cycloheximide at a concentration of 10.0 \mu g/\text{ml.}, two-thirds survived and one-half of all injected cells retained the ability to regenerate. Production, by absorption, of an intracellular level of antibiotic comparable to that obtained by microinjection would require 100\% uptake of the total cycloheximide content of a microchamber filled with a concentration of 1 \mu g/\text{ml.}

A complete description of this study requires photographs of regeneration and data too extensive for a brief summary. The complete description will therefore be published elsewhere. We feel that, even on the basis of this brief description, caution must be exercised in the interpretation of data based upon the use of cycloheximide concentrations above 1.0 \mu g/\text{ml.} In addition, even though the observations relating to regeneration are consistent with mechanisms based upon inhibition of protein synthesis, the tolerance of cells to the high injected concentration suggests that the toxicity of external cycloheximide may result directly from effects upon the cell membrane, and only secondarily from inhibition of protein synthesis.

We have observed the following effects of cycloheximide (Actidione) on the Oak Ridge and Rockefeller-Lindgren wild type strains of Neurospora crassa: 1. Detectable inhibition of growth occurs at a concentration of 0.1 \mu g/\text{ml.}, and a concentration of 0.5 \mu g/\text{ml.} causes more than 75\% inhibition of growth of standing cultures.

Hitchcock, S.E. and V.W. Cochrane. Effect of cycloheximide and actinomycin Don germinating conidio. In a study of the germination of conidio of wild type strain Em 5297a (ATCC#10816), the synthetic capacities of conidio incubated in minimal medium with and without a carbon source were investigated. Conidio were grown on Vogel's medium with 1\% glucose incubated in Vogel's liquid medium N with (called "germinating") 2\% glucose at a spore concentration of 10 \mu g/\text{ml.} wet weight.

Cycloheximide (Upjohn Co.) at concentrations of 1, 10, and 100 \mu g/\text{ml.} (0, 1, 1.0, 10.0 \mu g/\text{mg wet weight}) inhibits germination completely and inhibits the incorporation of L-leucine-U-C^{14}, phenylalanine-U-C^{14}, and proline-U-C^{14} into protein (hot TCA insoluble, hot NaOH soluble material). Inhibition at 1 \mu g/\text{ml.} was usually greater than 84\%. At 10 \mu g/\text{ml.} greater than 97\%, and at 100 \mu g/\text{ml.} greater than 99\%. The inhibitor had complex effects on the amino acid pools which have not been analyzed.

Figures 1 and 2 show the effect of cycloheximide on RNA synthesis in "germinating" conidio. When cycloheximide was added at the beginning of the incubation, germination was inhibited and RNA synthesis approximated that in "non-germinating" conidio (Figure 1). When the inhibitor was added after germination had begun, RNA synthesis continued at the control rate (Figure 2). Addition of cycloheximide at 30 minutes inhibited completely, while if added at 80 minutes, it halted germination after 20 minutes. It may be concluded that, while some RNA synthesis occurs in the absence of protein synthesis, continued protein synthesis is required for RNA synthesis at the rate found in germinating conidio.