THE EFFECT OF POLYENE ANTIBIOTICS ON PERMEABILITY IN NEUROSPORA CRASSA

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Several antibiotics and synthetic fungicides were examined during the course of an investigation on induced enzyme synthesis in the mold, Neurospora crassa. Fifty per cent growth inhibition was obtained with the polyene antibiotics, nystatin and amphotericin B at concentrations of 2.9×10^{-7} M and 5.6×10^{-8} M, respectively. Forty-eight hour mycelial mats grown in the absence of antibiotic rapidly lost weight when these concentrations of antibiotics were added (Figure 1). At low levels of nystatin (also amphotericin B) the decrease in weight began immediately and proceeded linearly. The average of 15 experiments showed a weight loss of 20 mg (range: 19-31 mg) after 5 hours incubation in the presence of excess antibiotic, representing a 25% decrease in the dry weight of 48 hour mycelial mats (range: 70-85 mg). This effect was apparently specific for polyene antibiotics since it has been obtained with all that have been tested (nystatin, amphotericin B, candidin, and filipin), occurred at extremely low concentrations, and was not duplicated by other antibiotics inhibitory for Neurospora, e.g. cycloheximide and viridin.

The nature of the weight loss obtained with nystatin was examined in greater detail (Figure 2). Concomitant with the decrease in weight there was a parallel appearance in the medium of compounds absorbing at 260 and 280 mμ. The ratio of optical densities at 280 and 260 mμ was essentially constant throughout the experiment with an average value of 0.468 (range: 0.434-0.500).

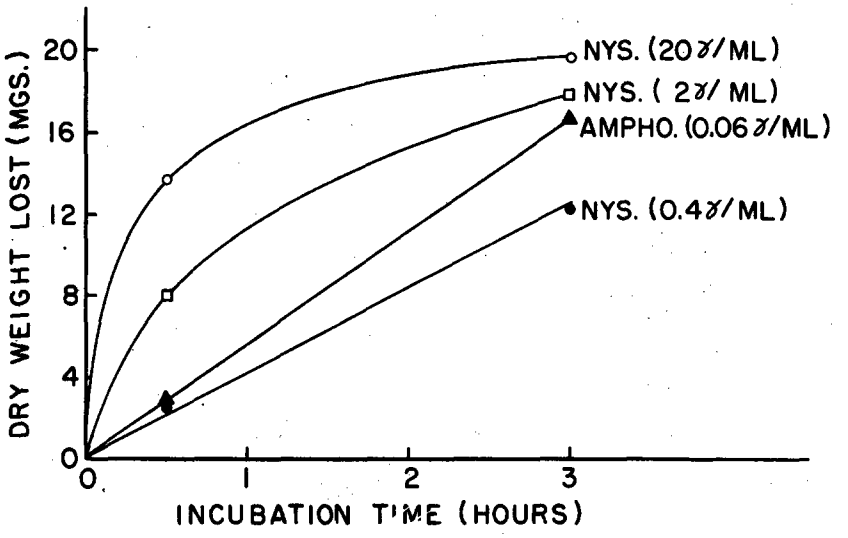

Figure 1. Effect of Nystatin Concentration on Dry Weight of Neurospora Mycelium. N. crassa was grown at 30°C as a stationary culture in 125 ml Erlenmyer flasks containing 25 ml of Fries' sucrose-salts-biotin medium. After 48 hours the mycelial mats were transfered to fresh medium, buffered with 0.02 M dimethylglutarate, pH 4.5, containing varying concentrations of antibiotic. After 0.5 and 3.0 hours incubation with shaking the mycelia from duplicate flasks were harvested by filtration on a Buchner funnel and dried in a vacuum dessicator over P_2O_5. Amphotericin B = (Ampho).

Preliminary experiments have indicated that this absorption was due mainly to purine and pyrmidine bases and nucleosides. Material which gave a positive reaction with the Folin protein reagent was also detected in the medium. The appearance of this material is plotted as "protein" (using crystallized bovine serum albumin as standard) in Figure 2 but it must be noted that a significant, although variable, fraction is dialysable. This may represent small peptides or possibly guanine and other bases which are known to interfere with this method of protein determination (Lowry et al, 1951).

After 4-5 hours the mycelial mats no longer lose weight but begin growing (Figure 2). The following observations indicated that was probably due to destruction of the antibiotic by photo-oxidation

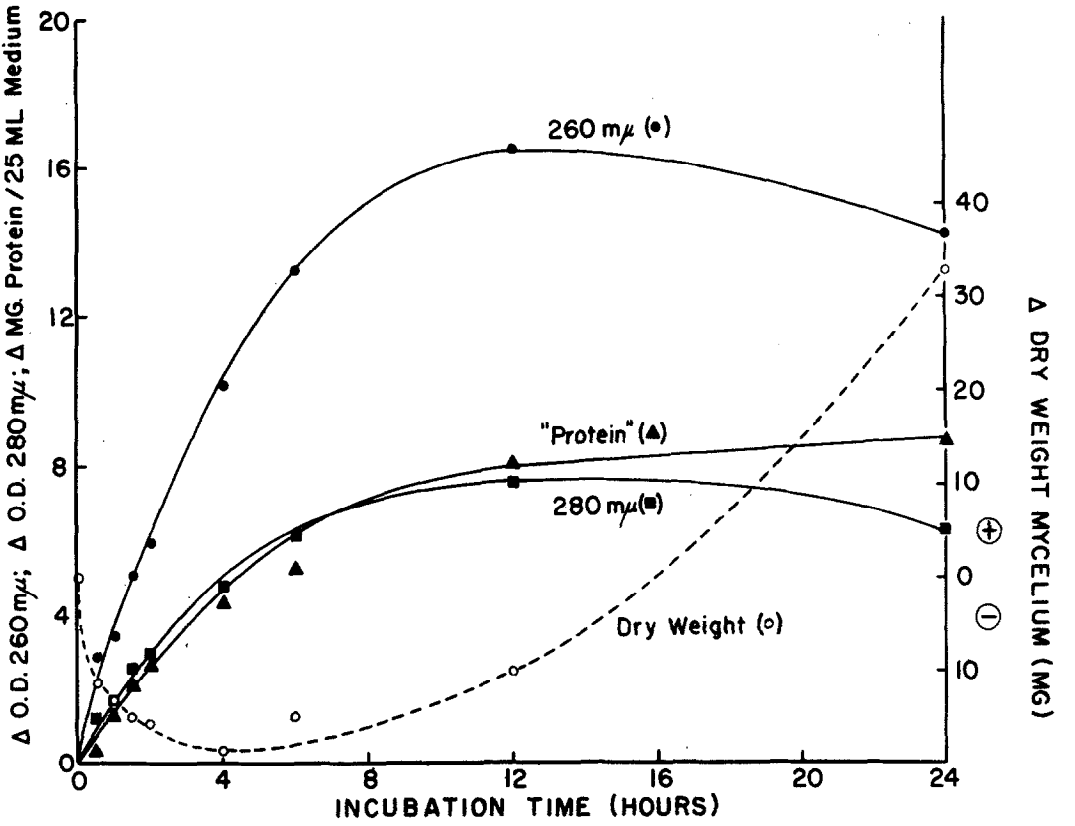

Figure 2. Changes Accompanying Nystatin-Induced Weight Loss in Neurospora Mycelium. N. crassa was grown at 30°C as a stationary culture in 125 ml Erlenmyer flasks containing 25 ml of Fries' sucrose-salts-biotin medium. After 48 hours the mycelial mats were transfered to fresh medium, buffered at pH 4.5 with 0.02 M dimethylglutarate, containing 0.80γ of nystatin per ml and shaken. At different times the mycelia from duplicate flasks were harvested by filtration on a Buchner funnel and dried over P_2O_5. The medium was analyzed for absorption at 260 and 280 mμ, and protein. The increase in absorption (expressed as optical density units/per 25 ml medium) or protein (mg/25 ml medium) is shown on the left ordinate. These values were essentially nil in zero-time control flasks. The change in dry weight (decrease or increase) relative to the weight of mats at the beginning of the experiment is shown on the right ordinate.

and not caused by the development of "resistance" (e.g. induction of an enzyme which inactivated nystatin). (1) Disappearance of the characteristic absorption spectrum of nystatin can be demonstrated under the conditions of the preceeding experiment in

growth media not containing mycelia. (2) Continued addition of nystatin after 4 hours prevents re-growth. (3) The addition of nystatin after 24 hours, at which time the mycelia actually weigh more than initially, again resulted in a decrease in weight paralleled by the appearance of 260 and 280 mμ absorbing material in the medium.

Polyene antibiotics have been reported to stimulate yeast respiration and glycolysis at low concentrations and to inhibit these metabolic parameters at higher concentrations. Such studies have focused attention on nystatin as a possible uncoupling agent (Drouhet et al., 1960) and inhibitor of glycolysis (Scholz et al., 1959). However, Lampen and co-workers were unable to demonstrate any in vitro effect of nystatin on enzymes of the glycolytic sequence in sensitive yeasts (Lampen et al., 1956; Scholz et al., 1959). The results presented here support the conclusion that an early and specific effect of nystatin (and other polyene antibiotics) is a permeability change resulting in loss of cell contents. The effects on respiration and glycolysis would then be secondary caused by leakage of some essential component.[1]

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[1] Addendum It is not yet known whether the 25% decrease in weight in 5 hours was manifested by all the cells comprising the mycelium or whether this value represented complete destruction of only 25% of the cells. Consequently, it is not possible to decide if the action of nystatin at low concentrations is reversible. This possibility is suggested by the observation that the mycelial mats will no longer lose weight, but increase in mass, at a time when appreciable destruction of the antibiotic can be demonstrated (Figure 2). Additional experiments have shown that this new growth was accompanied by the disappearance of 260 and 280 mμ absorbing material from the medium. Since a mycelial mat consists of a population of cells varying in age, it is also possible that nystatin is only effective on the fraction of cells which are growing. Anand and Davis have shown that protein synthesis is required for streptomycin-induced permeability changes in E. coli (Anand and Davis, 1960). While this manuscript was in review, a paper appeared by Marini et al., who, studying the effects of Na^+ and K^+ on nystatin inhibition of Saccharomyces cerevisiae, also concluded that the antibiotic produced an alteration in permeability. (Marini et al., 1961)

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