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 conidia incubated in minimal medium with and without a carbon source were investigated. Conidio were grown on Vogel's medium N with 1% glucose and 2% agar. Conidia were harvested at 7 days, washed, and incubated in Vogel's liquid medium N with (called "germinating") or without (called "non-germinating") 2% glucose at a spore concentration of 10 mg/ml wet weight.

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

Figurer 1 and 2 show the effect of cycloheximide on RNA synthesis in "germinating" conidia. When cycloheximide war added at the beginning of the incubation, germination was inhibited and RNA synthesis approximated that in "non-germinating" conidia (Figure 1). When the inhibitor was added after germination hod begun, RNA synthesis continued at the control rote for a short period and then leveled off and never attained the level of the control (Figure 2). Addition of cyclohcximide at 30 minutes inhibited germination 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 conidia.

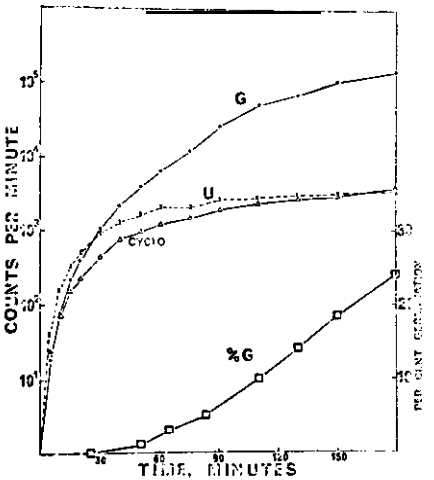

Figure 1. The effect of cycloheximide on RNA synthesis. Incorporation of uracil-2-C^{14} (5 μc/flask, 0.075 mM) into TCA insoluble material. G="germinating"; U="non-germinating"; CYCLO=cycloheximide, 100μg/ml; %G=percent germination of "germinating" conidia.

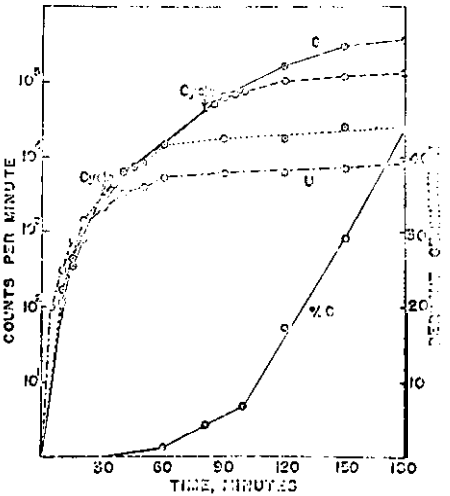

Figure 2. The effect of cycloheximide on RNA synthesis when added after the beginning of incubation. Cycloheximide was added to germinating conidia at 30 or 80 minutes. Experimental conditions and symbols are as described for Figure 1.

Table 1. Effect of actinomycin D on RNA synthesis and germination.

Actinomycin D (μg/ml)	0	1	10	25	100	250
% germination at 2.5 hrr.	40	40	38	40	--	23
CPM in TCA insoluble material at 0.5 hrs.	2,529	2,417	2,155	2,182	2,208	1,613
CPM in TCA insoluble material at 1.5 hrs.	32,092	32,056	32,498	28,053	28,549	16,630

Table 1 shows preliminary results of the effect of actinomycin D (gift of Merck, Sharpe and Dohme) on RNA synthesis and germination. Concentrations of up to 100 μg/ml had a small, if any, effect on RNA synthesis and germination. An extremely high level, 250 μg/ml, inhibited incorporation of uracil-2-C^{14} into TCA insoluble material only by 48.2% and germination by 57.5%. It was not determined if the actinomycin D was taken up by conidia.

This research was supported by NSF grant GE 1401. Department of Biology, Wesleyan University, Middletown, Connecticut 06457. Present address of SEH: Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106.