Baer, D. and P. St. Lawrence. Autoradiographic determination of the location of radioactivity in asci grown on some tritiated pyrimidines.

The intent of this project was to determine the period(s) of DNA synthesis in relation to the chromosomal events during ascus formation. Preliminary experiments were designed to test whether labeling in the nuclei of cultures maintained on various tritiated pyrimidines throughout the sexual cycle could be detected by autoradiography. The results demonstrate that, under these conditions, commercially available tritiated thymine, thymidine and deoxyuridine do not yield DNA with sufficient radioactivity to be useful for studies of DNA synthesis by autoradiography. The biochemical studies of Fink and Fink (1962 J. Biol. Chem. 237: 2289 and 2889) suggest an explanation of these observations.

Cultures of wild type ST74A were grown for 5 days at 25°C on synthetic crossing medium (Westergaard and Mitchell 1947 Am. J. Bot. 34:573) containing approximately: 0.05 mc/ml of either H^3-thymine (S.A. 6.6 c/mM, New England Nuclear) or thymidine-methyl-H^3 (S.A. 6.7 c/mM, New England Nuclear); or 0.03 mc/ml H^3-deoxyuridine (S.A. 1.3 c/mM, Schwarz). The position of the label on the H^3-thymine was unspecified but presumably some of the ring carbons carried tritium. Position of the label in deoxyuridine was unspecified but presumably C5 and C6 were tritiated. Portions of the mycelia were fixed after 11 and 49 hours of growth. Fertilization with wild type conidia was followed by fixation of asci and mycelium six days later. Standard radioautographic procedures were used, including treatment of some fixed material with DNAase or RNAase prior to covering with film.

The label in both mycelium and asci grown on the thymine compounds was light and uniformly distributed. No evidence for localization of grains over nuclei in the asci could be obtained from slides treated with the enzymes. Neither DNAase nor RNAase treatment appreciably reduced the amount of label/unit area: 1/3, at most, was removed by RNAase. Fink and Fink (ibid.) have shown that Neurospora rapidly demethylates exogenous thymidine and have suggested that, prior to incorporation into DNA, the thymine moiety is derived by methylation of uridine deoxynucleotide. Our inability to detect radioactivity in nuclei from cultures fed with tritiated thymine compounds and the failure of several workers (various conversations) to find thymidine mutants appear consistent with this suggestion.

Extensive labeling of both mycelium and asci was obtained with H^3-deoxyuridine, and the location of the grains in the film was equally dense over cytoplasm and nuclei. Treatment with DNAase did not reduce the number of grains/unit area significantly. RNAase treatment considerably decreased the amount of radioactivity, but no concentration of grains over the nuclei was revealed. It was concluded that most of the label was incorporated into RNA; the proportion (if any) incorporated into DNA was too slight to serve as an indicator of DNA synthesis against the heavy background of cytoplasmic radioactivity. R. M. Fink (1963 J. Biol. Chem. 238: 1764) has reported that some commercial H^3-uridine preparations seem to have most of the isotope at C5; methylation of this carbon would therefore yield unlabeled DNA. Possibly deoxyuridine-C6-H^3 would provide a means of labeling Neurospora DNA. A thymidylic acid mutant (if reparable) would be desirable. We shall try for one. - - - Department of Biology, San Diego State College, San Diego and Department of Genetics, University of California, Berkeley, California.