Johnson, T. E. **A method for obtaining high specific activity radioactive extracts from perithecia.**

One way of dissecting a biological process is by isolating mutants which are blocked at different points in that process. There mutants ore then used to determine what alterations hove occurred in the molecular components of that process.

Many mutants hove been isolated which block perithecial development as either the male or female component of a cross (review in Johnson, T.E., (1978) Genetics 88: 27-47). One very general ❑ ppmch to understanding the molecular alterations in these mutants is to determine what changer hove occurred in the proteins which are synthesized by those mutants. This report presents a reliable way to label perithecial proteins with radioactive amino acids. The proteins can then be displayed by one or two dimensional electrophoresis and autoradiography.

The desired maternal component is inoculated on crossing medium (Westergaard and Mitchell (1947) Am. J. Botany 34: 573-577) supplemented with 2% sucrose and appropriate growth requirements. Prior to inoculation the petri plates should be covered with filter papers so that the whole surface of the plate is covered (VWR grade no. 613 is especially suitable become the paper withstands long periods of wetting without shredding).

Conditions for growth and fertilization have been given (Johnson (1978) Genetics 88: 27-47). At appropriate timer after fertilization protoperithecia and perithecio can be isolated by scraping the surface of the paper with a blunt spatula. The mat is pressed dry, weighed and suspended in crossing medium with no sucrose (5 ml per plate harvested) and the desired radioactive label. The culture is then incubated on a rotary shaker for six hour. and harvested by filtration through a .6μ Millipore filter. The sample is

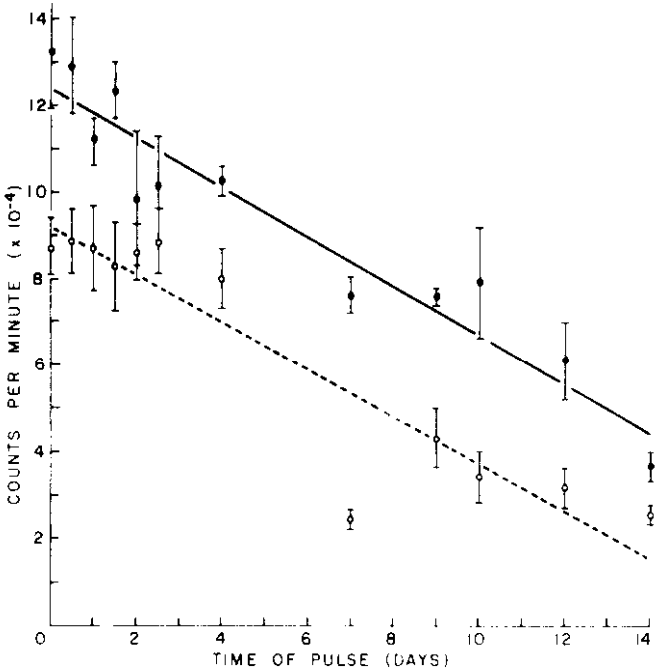

Figure I. Cultures were harvested and incubated, for six hours, total activity taken up i.-, overage of three repeats ± Standard Deviation,; total radioactivity incorporated into TCA precipitable counts (o-o-o).

then ground (Nasrallah and Srb (1973) Proc, No,. Acad. Sci. USA 70: 1891-1893), centrifuged at 5,000 g for 5 minutes and the supernatent frozen at -60° C. Before use, the sample is thawed and centrifuged again at 5,000 g for 5 minutes.

The amount of label incorporated into TCA precipitable material plateaus at about four hours. Labelled proteins can be obtained at any point in development by using this techniue; there is, however, a drop off both in uptake and in incorporation at later points in the development.3 program (Figure 1). Using ^{35}S-methionine (2.2μ Co, 700mC/mμ), I have obtained extracts containing as much as 1000 cpm/μg protein. This technique provider a very efficient uptake of label with minimum handling after labeling.

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