mersion of perithecia results in uptake via the ostiole.

The following is a description of our current procedure. Perithecia are collected in cross-sections made by spreading a conidial suspension of both parental strains on Westergaard's medium in petri plates. After the onset of ascus formation, before the beginning of ascus formation, perithecia are collected with the aid of fine forceps and transferred onto 4% agar. After removing any excess Westergaard's agar medium adhering, a flattened inoculating needle is used to transfer the perithecia to 10 x 75 mm test tubes filled with 1.5 ml of submersion medium. Usually 15 perithecia are transferred to each of two tubes. The perithecia in one tube are left submerged and the perithecia in the second tube are submerged for a specific length of time (ehrs. or days), then poured into a filter-lined funnel, washed with about 30 ml distilled water, and transferred to a slant of 2% purified agar. Perithecial contents are checked at intervals (usually 1, 2, and 3 days after transfer) to determine whether ascus formation proceeds normally.

The following parameters have been tested for their effect on perithecial development. a) Preliminary handling of perithecia -- when perithecia have been "cleaned" by quickly removing excess agar and by washing in distilled water both before and after submersion compared to perithecia not cleaned, no differences in ascus formation are observed. Thus, if care is taken to avoid dessication, the perithecia can be handled without ill effect. b) Perithelial stage -- in general, the older the perithecium, the better it survives submersion. When 2-3 day old material is submerged and subsequently transferred to agar, a few mature and forms spores, but the majority deteriorate before forming asci. Most 4-5 day old perithecia survive submersion and form ripe spores, but the frequency of ascus abortion is often higher than in unsubmerged perithecia. Perithecia that already contain asci, but no spores, usually survive submersion well. c) Submersion medium -- in most experiments involving several submersion media, distilled water was used as the control; and thus as a standard of comparison for evaluating the other media tested.

Following is a list of submersion media tested. In each case, perithecial contents were examined qualitatively to determine the presence or absence of detrimental effects, such as increased ascus abortion, variation in spore size or shape, or increased frequency of 5-spored asci. -- Best, subsequent perithecial development: Units: aminoid/1.05% NaCl, Squibb mineral oil; Good: 0.05% NaCl, 50 units aminoid/1.05% NaCl; Average: distilled water, liquid Westergaard's medium containing 0.2%, 2%, or 4% sucrose, liquid Westergaard's medium diluted 1:1 with distilled water, 5mM caffeine, 10 mM caffeine; Poor: liquid Westergaard's medium containing 8% sucrose, 8% sucrose solution, 0.05 M colchicine; Toxic: 0.1 M colchicine, 0.1 M phosphate buffer (pH 6.7), 0.3 M acetic acid buffer (pH 5.2).

Requirements for completion of perithecial development during continuous submersion are stricter than those for survival after temporary submersion. -- Good, comparable to an unsubmerged culture: Units: aminoid/1.05% NaCl, Squibb mineral oil, 0.05% NaCl; Average: distilled water, 50 units aminoid/1.05% NaCl; Poor, only a few spores form or that spores or asci are abnormal; liquid Westergaard's medium containing no sucrose, 8% sucrose solution, 5mM caffeine; little or no further development; liquid Westergaard's medium containing 2%, 4%, or 8% sucrose, liquid Westergaard's medium (2% sucrose) diluted 1:1 with distilled water, 10 mM caffeine.

Several interesting facts emerge. Although Westergaard's medium is a widely used crossing medium, continuous submersion of developing perithecia in liquid Westergaard's medium inhibits further development, even when 6-7-day-old perithecia, which already contain young spores, are submerged. Sucrose in the submersion medium also seems detrimental to further development. Also, once perithecia start forming asci, they seem to be self-contained, requiring no obvious source of nutrients (i.e., they will develop in distilled water) and little or no external oxygen (good development in mineral oil). Perithecia that have been submerged in distilled water for up to 7 days -- with no further development during submersion -- will, upon transfer to agar, resume development and form normal spores. There is, however, a very weak correlation between the length of submersion before transfer and the amount of ascus abortion. A six hour submersion seems to provide adequate uptake of the compound of interest and does not usually result in increased ascus abortion.

There is also a relationship between the age of the perithecium and the length of submersion tolerated. For example, perithecia which contain only sterile hyphae and crossters will tolerate a six hour submersion in liquid Westergaard's medium quite well; but if submerged for 24 hours, approximately 80% of the perithecia degenerate. Older perithecia, which already contain asci, will survive quite well after a 24-hour submersion in the same solution.

No differences in development were detected between perithecia transferred to purified agar and those transferred to agar containing Westergaard's medium. The advantage of purified agar is that subsequent hyphal growth and de novo perithecial formation are kept at a minimum.

Submersion may be a useful procedure for a variety of studies. It can be used as a means for effecting perithecial uptake of nutrients, inhibitors, etc., and would be especially suited for compounds that are too unstable to be added directly to a crossing medium, or, conversely, for compounds that inhibit crossing per se. Results from continuous submersion may provide insight into the nutrients, etc. necessary for in vitro development of isolated asci. (This research was supported by a Predoctoral Training Grant, T3 GM-01035, from the National Institute of General Medical Sciences, USPHS, and by Grant GM-12953 to A.M. Sch.) From the National Institute of General Medical Sciences, USPHS.) The Section of Botany, Genetics and Development, Cornell University, Ithaca, NY 14853.

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

[Image of 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 are then used to determine what alterations have occurred in the molecular components of that process.

Many mutants have 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 changes have 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 supplemented with 2% sucrose and appropriate growth requirements, and pressed paper 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 time after fertilization protoperithecia and perithecia 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 hours, and harvested by filtration through a 0.8 Millipore filter. The sample is
Figure I. Cultures were harvested and incubated, for six hours, total activity taken up i.e., average of three repeats ± Standard Deviation; total radioactivity incorporated into TCA precipitable counts (o-o-o).

then ground (Nasrallah and Srb, 1973 Proc. Acad. Sci. USA 70: 1891-1893), centrifuged at 5,000 g for 5 minutes and the supernatant 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 technique; there is, however, a drop off both in uptake and in incorporation at later points in the development (Figure 1). Using 35S-methionine (2.2µ Ci, 700µCi/ml), I have obtained extracts containing as much as 1000 cpm/µg protein. This technique provides a very efficient uptake of label with minimum handling after labeling.

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The desired maternal component strain is inoculated on day 0 into petri plates containing standard Westergaard’s crossing medium (Am. J. Bot. 1974)34; 573-577 supplemented with 2% sucrose and 4% agar.

On the fifth day (or whenever protoperithecia appear) 2 ml of a suspension of conidia of 105/ml or greater is pipetted over the plate and spread gently with a spreading bar if necessary, if large amounts of condia present on the ridges of the plate, there can be removed with tissue wetted with alcohol.

Protoperithecia can be harvested at any time, by gently scraping the surface with a broad, blunt spatula that has a burr turned under. This preparation is relatively pure, but additional purification can be obtained by chopping the material in an Omnimixer for twenty seconds at 2,000 rpm. The protoperithecia are then allowed to settle out in a graduated cylinder and collected and concentrated on a filter apparatus.

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Efficiency of Cell Disruption with the Braun Homogenizer

<table>
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<th>time (sec) of homogenization</th>
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Homogenizations were performed in 30 sec pulses followed by 30 sec rests.