I have found the first of the two methods to be highly satisfactory for obtaining mycelial pads when analysis of portions of each pad is desired. **Biology Department, Rochester Institute of Technology, Rochester, New York 14623.**

Hedman, S. C. Determination of total phosphorus in Neurospora extracts.

Often one desires to ascertain the total phosphorus content of various Neurospora extracts. The following method has been found applicable for a wide variety of such extracts. This method incorporates various features of previously published procedures as well as some new modifications. There are two parts to the procedure: acid hydrolysis and phosphate determination.

**Acid hydrolysis:** 0.2 ml of extract (containing 1-10 μg of phosphorus) is placed in an acid-cleaned 15 x 150 mm Kimax test tube. 0.3 ml of 5 N H₂SO₄ and 0.9 ml of H₂O are added. The contents are slowly heated over a Bunsen burner until dense white fumes of SO₃ are given off. At this point, the contents of the test tube may be dark-brown to black in color. After cooling the mixture, 0.1 ml of 2 N HNO₃ is added and heat is applied until SO₂ is again given off. This HNO₃ treatment is repeated until the contents of the test tube are colorless. The volume is then brought to 1.5 ml by the addition of H₂O and the tube is heated in a 100°C water bath for 5 minutes to hydrolyze pyrophosphates. An acid-cleaned glass marble is placed over the top of the test tube to prevent excessive evaporation.

**Phosphate determination:** To 1.5 ml of hydrolyzed extract ore added 1.2 ml of phosphate reagent. The phosphate reagent is made as follows: (a) Stock solution: 50 g of ammonium molybdate.4H₂O are dissolved in 400 ml of 10 N H₂SO₄ with constant stirring. After all is in solution, the volume is brought to 500 ml with additional 10 N H₂SO₄. This stock solution can be stored for several months at room temperature. (b) Preparation of reagent: The phosphate reagent must be made up fresh for each series of assays. To make 20 ml of such reagent, 2.0 ml of stock solution are added to 14 ml of H₂O containing 1.0 mg of FeSO₄.7H₂O. After the ferrous sulfate is in solution, the volume is brought to 20 ml with distilled water.

After five to ten minutes, the absorbance of each tube is read at 710 μm in 1 ml cuvettes of 1.0 cm path length. A reagent blank is used as a reference. Under these conditions, linearity is observed between absorbance and phosphorus content over the range of 1-10 μg. 10 μg of phosphorus routinely gives an optical density of 0.464 ± 0.010.

The phosphorus determination by itself can also be utilized to determine the total orthophosphate content of, for example, when assaying for phosphatase activity. The following compounds do not interfere with this method: tris buffer (0.2 M), trichloracetic acid (20% w/v), bovine serum albumin (400 μg/1.5 ml), CH₃OH, CH₂OH₃, Cleland's reagent (10⁻⁴ M), or sucrose (0.3 M). **Department of Biology, University of Minnesota, Duluth, Duluth, Minnesota 55812.**


During experiments in which the rate of uptake of different sugars by conidia of a number of different strains of Neurospora crassa was to be estimated and referred to their protein content, a quick and reliable method for measuring the protein content of ungerminated conidio was needed. For technical reasons, we first tried to measure their protein content directly by applying a modification of the Biuret method as described by Beisenherz et al. (1953 Z. Naturforschung 8b: 555) to whole conidia.

Individual steps of this modification are as follows: (1) Prepare conidial suspensions of 5 x 10⁷ conidia/ml. (2) Precipitate the protein in 1 ml of the conidial suspension with 0.2 ml 50% TCA, shake well and spin down. (3) Wash two times with a mixture of 3 parts ethyl alcohol and one part ethyl ether and a third time with ethyl ether to remove carotenoid color and other lipids. (4) Let the ether evaporate from the pellet and resuspend in 1 ml Biuret reagent. (5) Incubate for 30 min. at room temperature on a shaker. Centrifuge and transfer the supernatant into a 1 cm cuvette. (6) Read the absorption at 546 μm. Add a small amount of KCN powder to the cuvette, stir and wait 1-2 min. until the remaining absorption is constant, and read again. The difference in absorption is due to the protein in the solution (Aₚ). (7) Measure the absorption of the Biuret reagent alone before and after addition of KCN. The difference in the blank (Aₜ) is to be subtracted from Aₚ. (8) Refer Aₜ to a calibration curve, obtained for bovine serum albumin to obtain mg protein/ml.

Applying this procedure and increasing the incubation time at room temperature from 30 min. to 120 min., a marked increase of the resulting absorption was observed (Fig. 1, curve a). This finding was thought to indicate that not all of the protein in a whole conidium was available freely to the Biuret-reagent. This was confirmed by increasing the temperature of incubation up to 50°C, when a further increase in absorption was observed (Fig. 1, curve b). Neither elongation of incubation-time up to 120 min. nor increase of incubation-temperature up to 50°C had a significant effect on absorption of calibration samples containing bovine serum albumin.

Applying this procedure to 5 x 10⁷ conidia (5 x 10⁷/ml) after incubation with the reagent for different periods of time.

In studies of enzymes induced by lactose and by galactose in Neurospora, it has become apparent that growth conditions must be carefully controlled, and that shattering cultures containing a single carbon source can provide reproducible conditions well-suited to such studies. The ideal carbon source should contain no repressive interference with the induction process. Glycerol is suitable for such studies, but wild type strains are quite variable in their ability to grow on glycerol under the required conditions. The isolate 105-L5-A (formerly designated L5D, Bates and Woodward 1967 Neurospora NewsL. 12:11) shows greatly improved growth on glycerol when compared with STA4. Crosses of this isolate to wild type 74-OR8-1a (Bates 1967 Genetics 56:543) yielded a variety of isolates with improved glycerol growth characteristics, although initial selection was for lactose growth. Two of these were crossed (211-L5-o x 341-8A) and an isolate designated 41-1-L5-A was obtained. This isolate has been used for all subsequent glycerol growth studies.

Growth conditions are: rotary shaking, 3/4 inch radius, 150 cycles per minute, 30 ± 0.5°C, 0.18 M glycerol, Vogel's medium, 200 ml in 500 ml Erlenmeyer flasks, mounted at a 30 degree angle. The Vogel's medium is autoclaved at 2 x concentration, and the carbon source is autoclaved separately in 100 ml water. The inoculum is 106 conidia per ml medium. Under these conditions, growth is linear for 90 hours (yielding ca. 1.5 g dry weight), and comparisons are made by harvesting at 48 hours (yielding ca. 0.7 g dry weight). The rate of growth with glucose under these conditions is ca. two times the rate obtained with glycerol.

In comparison with 411-L5-A, taken as 100%, growth of some wild type strains on glycerol can be grouped in the following way: STA4 and RL-A, 33-36%; ST73a, RL-o and Em-o (FGSC#691), 58-68%; Em-o (FGSC#692), 103% (all based upon total mycelial dry weight at 48 hrs). RL-A and RL-o are Rockefeller-Lindgren isolates obtained from J. F. Wilson. It is apparent that a mating type shows better glycerol growth than does A for all three strains, except for 411-L5-A. When grown on sucrose, two strains differed considerably (±10%) in total growth is observed among these strains. Another distinguishing characteristic is the orange pigment which occurs under these growth conditions in an inverse relationship to ability to grow on glycerol. The Em-o and 411-L5-A cultures show no evidence of this pigmentation.

Among the isolates obtained along with 411-L5-A, there was a marked correlation between ability to grow on glycerol and reduced production of conidia. For example, 411-L5-A producer only 30-50% of the conidia produced by STA4 when grown and harvested under the same conditions. This characteristic is not necessarily associated with glycerol growth is shown by Em-o, which conidiates more abundantly than STA4, but which grows well on glycerol. Crosses designed to combine the glycerol growth characteristics with amino acid and inositol requirements are now in progress. The 411-L5-A isolate producer abundant protoperithecia on Westergaard's synthetic cross medium, and up to 90% spore viability, but the mature perithecia apparently have low internal pressure, and discharge ascospores weakly.

It appears that the 411-L5-A amino acid auxotrophs have very similar glycerol growth characteristics, but these traits have not been completed. Such characteristics would allow very precisely controlled studies of incorporation of labeled amino acids during induction studies. If these isolates appear to be potentially useful to other workers, the set of cultures will be deposited in the Fungal Genetics Stock Center. (Supported by NSF Grant GB 5189).