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Abstract
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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 NH2SO4 and 0.9 ml of H2O are added. The contents are slowly heated over a 50°C water bath for 5 minutes. When the mixture is colorless, 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 are 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 pg. 10 µg of phosphorus routinely gives an optical density of 0.464 ± 0.010.

The phosphate determination by itself can also be utilized to determine the total orthophosphate content, as, for example, when assaying for phosphatase activity. The following compounds do not appear to interfere with this method: tris buffer (0.2 M), trichloracetic acid (20% w/v), bovine serum albumin (400 µg/1.5 ml), C2H5OH, sucrose (0.3 M).


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. Naturforsch 8b: 555) to whole conidia.

Individual steps of this modification are as follows: (1) Prepare conidial suspensions of 5 x 10^7 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 (A_p). (7) Measure the absorption of the Biuret reagent alone before and after addition of KCN. The difference in the blank (Ab) 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.

In additional experiments, conidia were therefore disrupted either by treating them 1 to 3 times with the X-press (AB BOX, Box 235, Nacka 2, Sweden) with a 25 ml volume operated at -25 to -35°C at ca. 2000 kg/sq cm, or by smashing them with glass beads (d = 0.45-0.50 mm) for 5 to 10 minutes in a homogenizer.

![Fig. 1](image)

Values for whole or disrupted conidia (5 x 10^7/ml) after incubation with the reagent for different periods of time.