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 H_2SO_4 and 0.9 ml of H_2O are added. The contents are slowly heated over a &men burner until dense white fumes of SO_3 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 HNO_3 is added and heat is applied until SO_3 is again given off. This HNO_3 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 H20 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.$4H_2O$ are dissolved in 400 ml of 10 N H_2SO_4 with constant stirring. After all is in solution, the volume is brought to 500 ml with additional 10 N H_2SO_4. 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 H_2O containing 1.0 mg of $FeSO_4.7H_2O$. 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 mµ 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. 10µg 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), ChC13, C_2H_5OH, Cleland's reagent (10^{-4} M), or sucrose (0.3 M). ▪ ▪ ▪ Department of Biology, University of Minnesota, Duluth, Duluth, Minnesota 55812.