Kulaev, I. S. and V. I. Melgunov. Determination of phosphorous in N. crassa extracts.

A method of total phosphorous determination in Neurospora extracts hor been described by Hedman (1969 Neurospora Newsl. 14: 10), who suggested the use of a method for total orthophosphate content estimation. However, like all other colorimetric methods of phosphate determination in the aqueous phase, this one seems to be liable to error arising from some disturbing factors (see Berenblum ond Chain 1938 Biochem. J.32: 295). besides which, the time of interaction between molybdote and phosphorous compounds in solution was very long (5-10min). Thus, the total orthophosphate content will be overstated because of the well-known catalytic effect of molybdote on the hydrolysis of organic phosphates. Therefore, we wish to turn Neurosporologists' attention to another, more advantageous, procedure of phosphate determination.

The method adopted in our laboratory is bored mainly on Weil-Malherbe and Green (1951 Biochem. J.49: 286) and Martin and Doty (1949 Analyt. Chem. 21: 965) modifications of the extraction method of phosphate estimation introduced by Berenblum and Chain (1938, ibid.). The solutions used ore: (1) mixture of isobutonol-benzene (1:1, v/v). (2) 5% ammonium molybdote in 4N H_2SO_4, prepared fresh doily by dilution of stock solution of 10% ammonium molybdote in 8 N H_2SO_4. (3) Stock solution of stannous chloride; 10 g $SnCl_2$ dissolved in 25 ml conc. HCl, kept in o brown glass-stoppered bottle at 0°C. (4) Dilute stannous chloride solution; 0.25 ml conc. solution diluted to IO ml with 1 N H_2SO_4 (must be made up fresh when required). (5) Acid ethanol; 10 ml conc. H_2SO_4 + 490 ml absolute ethanol.

Procedure: If the solution to be tested is strongly acid or alkaline, it must first be neutralized to pH 7-8 with NaOH or HCl. Then odd 6 ml irobutonol-benzene mixture and 1 ml 5% ammonium molybdate in 4 N H_2SO_4 to the test solution mode up to 5 ml in a glass-stoppered test tube. Shake it immediately for 15 sec. With a fine-tipped pipette connected too suction flask, discard the aqueous bottom layer as completely as possible. Then odd a pinch of anhydrous Na_2SO_4 to the test tube and shake it until the extract is cleared of any emulsified droplets. By means of a syringe pipette, withdraw 2 ml and transfer to a second test tube. Add 2 ml acid ethanol, 0. 1 ml dilute $SnCl_2$ solution and mix by shaking. After 10 min, the intensity of blue color may be deter-

mined either by an ordinary colorimeter with a red filter or by means of spectrophotometry at 650 nm in cuvettes of 1.0 cm path length. Construct a calibration curve in the usual way. The stock phosphate solution required for comparison is prepared as follow: 2.193 g KH_2PO_4 in 500 ml water (= 1 mg P/ml).

Under the conditions described, linearity is observed between absorbance and phosphorous content over the range of 1-25 pg. 21.2 µg of Phosphorous gives an optical density of 1.000 ± 0.010. The most reliable results are obtained in the range of 1-10 µg. The phosphate determination can also be utilized to estimate the total phosphorous content and the content of acid-labile phosphates. The rum of labile phosphates and orthophosphate is determined in a cooled neutral hydrolysate of the romple after 10 min hydrolysis with on equal volume of 2 N HCl in a boiling water bath.

For the determination of total phosphorous the romple content must be incinerated by the addition of 0.2-0.3 ml of 57% $HClO_4$ and the subsequent heating of the romple an a special electric stove equipped with a contact thermometer and a duralumin disc with rockets (about 50 in number) for the test tuber. For the first I-2 hours, the heating is carried out at 110-120°C, until the water has completely evaporated. Then the temperature is raised to 170-180°C and incineration proceeds to obtain a fully colorless solution. The incinerated sample is mode up to I-2 ml, approximately, by adding water and the test tube is heated in a boiling water bath for 10 min to hydrolyze pyrophosphates formed during incineration. The determination of phosphate in the neutralized romple is carried out as usual.

The advantages of this method are related to the discording of the aqueous layer, the absence of the non-specific development of blue color in the control and the reduced contact between the molybdate reagent and the labile phosphate bonds (15 sec), all of which were accurately stated in the papers of the authors cited above.

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