

A method for the increased incorporation of P³² into Neurospora DNA

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A method for the increased incorporation of P³² into Neurospora DNA

Abstract

Incorporation of P³² into DNA

Richman, N. and S.K. Dutta. A method for the increased incorporation of P^{32} into *Neurospora* DNA.

the uptake of radioactive phosphate in minimal media is very low. Apparently the uptake of P^{32} by the growing fungal mycelia is greatly inhibited by the phosphate already present in the Vogel's minimal medium.

In order to increase the amount of P^{32} incorporation into the DNA molecules of *Neurospora*, studies were conducted to determine the minimal requirements for phosphate. Phosphate-less Vogel's minimal medium was prepared and varying amounts of KH_2PO_4 were added. The ability of *Neurospora* mycelia to grow in different concentrations of phosphate was tested. The minimal quantity of phosphate necessary for growth of the fungal mycelia was found to be 0.05 g. percent. This compares with 0.5 g. percent in the normal Vogel's medium.

Labelling of the DNA was accomplished by adding 2 mc of $Na_2HP^{32}O_4$ (sterile solution; Nuclear Consultants, St. Louis, Mo.) per liter of medium. A heavy fungal inoculum was introduced and the mycelia were harvested after 14-16 hours growth.

The DNA was isolated by the method of Marmur (1961 J. Mol. Biol. 3:208) as modified by us (1967 *Neurospora* Newsl. 10:26). Phenol extraction was found to be superior to chloroform for the separation of proteins. Not only does the use of phenol afford a more simplified procedure but its use apparently lessens the shear forces acting to degrade DNA and a higher molecular weight DNA results (Josse and Eigner 1966 Ann. Rev. Biochem. 28:789).

The purity of DNA was determined by UV-spectrophotometry and $CsCl_2$ density gradient centrifugation. Counts of purified DNA solutions were taken with an end-window GM tube (window thickness of 2.7 mg/cm^2). The counts per minute (cpm) per μg DNA for Vogel's medium was 126, while the medium with 0.05 g. percent KH_2PO_4 gave a net cpm/ μg DNA of 4418. Although the half-life of P^{32} is only 14.22 days, the high specific activity obtained by our procedure allows the use of the labelled DNA for several days. Research supported by Texas Southern University Faculty Grant No. 16876. • • • Department of Biology, Texas Southern University, Houston, Texas 77004.

During the course of studies of a nucleic acid hybridization in fungi, it was necessary to label *Neurospora crassa* DNA. The most convenient radionuclide for our purposes, since we were not employing a liquid scintillation detection system, was found to be Phosphorus-32. Earlier attempts by Dutta, McWhorter and Woodward (1965 *Neurospora* Newsl. 7:9) suggested that