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

During the course of studies an nucleic acid hybridization in fungi, it was necessary to label Neurospora crassa DNA. The mast 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 the uptake of radioactive phosphate in minimal media is very law. 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 far 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 far the separation of proteins. Not only &es the use of phenol afford a more simplified procedure but its use apparently lessens the shear farces acting to degrade DNA and a higher molecular weight DNA results (Josse and Eigner 1966 Ann. Rev. Biachem. 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 far several days. Research supported by Texas Southern University Faculty Grant No. 16876. ▪ ▪ ▪ Department of Biology, Texas Southern University, Houston, Texas 77004.