Gillie, O. J. **Methods of making enzyme extracts and amino acid pool extracts from Neurospora grown on solid media.**

Methods have been devised for extracting enzymes and amino acids from mycelium grown on solid media in order to be able to study biochemical events which take place in periodic or irregularly growing mutants. Such procedures are desireable as certain types of growth behavior ore found on solid media which cannot be recognised in liquid media.

Growth methods: Straight-ended growth tubes (Brown and Gillie 1963 NN#4: 19) from which mycelium and agar could be easily removed or culture trays (Pyrex ovenware lids 13 x 21 x 2 1/2 cms covered with specially made aluminum lids) ore filled with 0.3% agar (Difco Bacto-Agar) medium after dry sterilization. Tubes are inoculated in the usual way and trays are best inoculated at 1 cm intervals. After growth has proceeded along most of the tube or tray, mycelium and agar ore removed from the tubes with a suitably flattened and bent wire and from the trays with a specially shaped stainless steel chopper. Using these implements, sections of mycelium can be readily cut at any desired distance from the growing part or point of inoculation.

Extraction of mycelium: To separate the mycelium from the agar, the sections are placed on a piece of fine cotton (muslin which has many loose fibers is not recommended for this) and after gathering in a fold of the material the sample is squeezed by twisting the material All the water and most of the agar in the sample is removed by this procedure. The sample is now scraped off the material and frozen.

Enzyme analysis: The sample is best freeze dried after which it may readily be powdered and then homogenized with buffer in a around glass homogenizer.

Amino acid analysis: The fresh or frozen or freeze-dried sample is mixed with 3.6% perchloric acid and heated in a boiling water bath for 5 minutes. This extracts amino acids and nucleic acids (5-10% of the dry weight appears to be extracted as ribo-nucleotides). These *extracts* may be *spotted (10 µl* spots give best results; if the *spots* are greater than 25 *µl* the *acid* interferes with the separation) and subjected to high voltage electrophoresis for 1 hour at 78 volts/cm, pH 3.4. After development of the papers, aspartate, glutamate, argininosuccinic acid, orginine ond ornithine form discrete spots, lysine and histidine form a spot together and argininosuccinic acid anhydrides (B and C form) also form a spot together but are separate from Z substance, a degradation product of argininoruccinic acid formed during the extraction. The remaining amino acids form a single 'neutral' spot which may be further separated by electrophoresis for 3-4 hours. The spots may be developed with ninhydrin and eluted quantitatively after treatment with copper solution according to the method of Bronk and Fisher (1956 Biochem. J. 64: 106, a modification of the method of Harris et al. 1954 Ann. Human Genet. 19: 196).

Approximately IO-15 mg of wild type mycelium per ml of 3.6% perchloric acid gives satisfactory spots, but argininosuccinic acid accumulations could be identified in as little as 100 µgm of material in 0. 1 ml 3.6% perchloric acid from arg-10 mutants grown on low concentrations of arginine. Quantities of mycelium as small as this are difficult to weigh and samples must be token from the mycelium and acid mixture for spotting before mycelium is separated on a pre-weighed membrane filter for weight determination.

Other methods of extraction of mycelium for enzyme or amino acid analysis have been found to involve excessive dilution of the sample in order to break up the agar gel. The method described works best with 0.3% agar, although 1% (or even 2%) agar may be used. The latter is not recommended as the extra thickness of the agar makes much greater pressures necessary which moy cause the threads of the material to expand and allow some of the mycelium through. When complete medium is used it is better to increase the agar concentration to 0.6%, as otherwise it does not gel properly, presumably due to the acidity of this medium.

Using methods of this kind, it has been possible to investigate periodic growth of arg-10 mutants on low concentrations of external orginine and to show higher accumulation of argininosuccinic acid in org-IO mutants grown on solid medium with low external orginine concentrations as compared with high external orginine concentrations; this change being associated with a 4-fold repression of arginase, a 7-fold derepression of ornithine transcarbamylase, a 5-fold derepression of argininosuccinase and a 4-fold derepression of *argininosuccinic acid synthetase*. - - - Deportment of Genetics, University of Edinburgh, Edinburgh, Scotland.