Wild type Neurospora crassa (74-OR-A) was grown on 1% lactose for 5 days and the mycelia were filtered out on a Buchner funnel. The mycelia were extracted with 10 ml of 0.01 M Na phosphate, pH 7.5, per g of wet weight. The mycelia were homogenized in an Omni-Mixer, sonicated, and then stirred for 2 hrs at 4°C. After centrifugation at 20,000 × g for 20 min, the supernatant was used as crude extract. The growth medium after filtration was concentrated by dialysis against dry sucrose. Samples for electrophoresis contained ca. 0.25 mg protein.

Crude extract gave reactions with ONPG at three distinct sites on the gel. The 7.5 enzyme had an Rf of 0.046, the 4.2 enzyme had an Rf of 0.250, and a third form of the enzyme had an Rf of 0.150. When the growth medium was electrophoresed, activity appeared at either one or two sites, depending upon the age of the culture. Medium from a young culture showed only the new form of the enzyme (pH 4.5) while medium from an old culture contained both the 4.2 and 4.5 forms with a predominance of the former. Between these two extremes there were gradations in the proportions of the two forms. This work supported in part by the NIH Training Grant in Genetics (T01-GM01316) to Florida State University. - Genetics Laboratories, Department of Biological Science, Florida State University, Tallahassee, Florida 32306.

Morgan, D. H. The assay of arginase.

Many methods of arginase assay in various organisms have been published. The following procedure has been found to work well with crude extracts of Neurospora.

Frozen mycelial pads are ground in a chilled mortar with glass powder and 5-10 times their weight of sodium hydroxide buffer containing manganese chloride (0.005 M) and dithioerythritol (0.002 M). An -SH reagent is possibly superfluous in undialysed extracts but has been found to stabilize the enzyme during dialysis.

A reaction mixture consists simply of 0.2 ml of enzyme and 0.3 ml of 0.3 M arginine. The arginine solution is adjusted to pH 9.5 and final pH in the mix is about 9.2, the arginine itself providing adequate buffering. Incubation is at 37°C, the enzyme being pre-incubated at this temperature for 10-15 min before arginine addition. The reaction is stopped with 4.5 ml of 2% TCA, zero-time blanks being stopped before arginine addition. Ornithine estimation (see below) is carried out on 0.5 ml samples of the stopped reaction mix. (When it is desired to use lower substrate concentrations and therefore to detect lower levels of ornithine, to stay in the linear region dilution with TCA is reduced or avoided altogether by stopping with the acid ninhydrin reagent used for the ornithine estimation.)

The estimation of ornithine in assaying for arginase or acetyl-ornithine/glutamate transacylase. The method of ornithine estimation used by Vogel and Bonner (1956 J. Biol. Chem. 218:97) for the assay of acetylornithinase is also applicable to the assay of arginase and acetylornithine/glutamate transacylase. It is quicker than the commonly-used method of Chinor (1952 J. Biol. Chem. 199: 91) and the ninhydrin mix used (made up in 0.4 ml citric acid and methyl cellulose) is pleasant to deal with than that of Chinor (6 M phosphoric acid and glacial acetic acid). The optimum boiling time for arginase samples is 25 min. Both glutamate and arginine give rise to a deep blue color after the final addition of NaOH. This persists until the samples are subjected to vigorous Vortex mixing (30-60 sec) when it disappears, revealing the stable golden-brown color which is read at 470 μm. It is necessary to read against no-ornithine blanks containing appropriate quantities of arginine or glutamate, both of which give appreciable blank values. Sensitivity is about three-fold lower than with Chinor at 0.4 μmoles of ornithine per OD unit at 470 μm in the presence of 9 μmoles arginine per sample. The reaction is linear at least up to 0.5 μmoles ornithine per sample.

This work was supported by the Gosney Fund, California Institute of Technology, The hospitality and encouragement of N. H. Horowitz was greatly appreciated. - Genetics Laboratories, Department of Biological Science, Stanford University, Stanford, California 94305.

Flavell, R. Comments on assay methods.

The following assays have been used for Neurospora as reported by Flavell and Fincham (1968 J. Bacteriol. 95: 1063). Any modifications used since then are noted after the relevant assay. The original references to the assays are given in the paper mentioned above.

Isocitrate lyase, E.C. 4.1.3.1.; but with 15 μmoles of DL-isocitrate.
Malate synthase, E.C. 4.1.3.2.; but with 0.04 μmoles of Acetyl C oA.
Phosphoenolpyruvate carboxykinase, E.C. 4.1.3.2.; but with 0 μmoles phosphoenolpyruvate.
Citrate synthase, E.C. 4.1.3.7.; but with 0.04 μmoles of Acetyl CoA.
Acetyl CoA synthetase E.C. 6.2.1.1.
Malate dehydrogenase, E.C. 1.1.1.37.
NADP-linked Isocitrate dehydrogenase, E.C. 1.1.1.42.
NAD-linked Isocitrate dehydrogenase, E.C. 1.1.1.41.; but with 8 mM DL-isocitrate and 1 mM NAD.
Fumarate hydratase, E.C. 4.2.1.2.

The following assays have also been used without modification of the method cited in the reference following each enzyme:
Succinate dehydrogenase, E.C. 1.3.99.1. (King 1963 J. Biol. Chem. 238: 4032)
- Department of Biological Sciences, Stanford University, Stanford, California 94305.