Hall, D. O. and H. Baltscheffsky. Rapid preparation of Neurospora mitochondria.

The preparation of mitochondria from Neurospora usually requires quite drastic treatments, involving the use of micro-mills, glass beads, sand or disruption of protoplasts, besides requiring from 2 to 12 hours time after harvesting. We have developed a method for preparing mitochondria in 30 minutes using a combination of three new techniques; viz., collection in a nylon bag, disruption with a glass homogenizer and rapid centrifugation.

N. crassa wild type strain Y74A (Genetics Institute, Copenhagen) was grown from conidia on Vogel's complete medium as described by Hall and Greenawalt (1964 Biochem. Biophys. Res. Commun. 17:565). Conidia from a slant grown at room temperature for 1-2 weeks were suspended in 10 ml of water and used as inoculum for 500 ml of Vogel's medium in a 2 l. Erlenmeyer flask. The culture was then grown for 14-16 hours at 30°C.

Working at CC, the hyphal mat was collected by pouring the culture through a nylon bag (double layer, mesh about 50 per cm: P. S. Nobel, Pl. Physiol., in press). The mat was resuspended in 200 ml 0.6M sorbitol, cut up with scissors, poured through the nylon bog again and then resuspended in 200 ml of a preparation medium consisting of 0.25M sucrose, 0.005M EDTA and 0.3% crystalline bovine serum albumen. The hyphae were then homogenized in 2 batches in a fairly loose-fitting, medium-coarse, ground glass Wicklund homogenizer (130 ml volume of ground glass stem and 230 ml total volume; Wicklund Glasinstrument, Idungatan 7, Stockholm, Sweden). A total of 5-6 strokes wwas required to break the hyphae and obtain an even suspension. The suspension was poured through the nylon bag to remove whole cells, cell walls, etc., and then centrifuged at 10,000 x g for 5 minutes. The sediment, which contained the mitochondrio, was gently resuspended in 100 ml of preparation medium using a Ten-Bmek ground glass homogenizer (A.H.Thomas and Co., Philadelphia). A further centrifugation at 10,000 x g for 5 minutes and resuspension of the sediment in about 3 ml of preparation medium (-EDTA) gave a mitochondrial fraction containing about 20 mg protein/ml. The addition of a short (1 minute) 2,000 x g centrifugation and/or the elimination of the second 10,000 x g centrifugation may be used to give cleaner and/or faster preparations.

These mitochondria exhibit oxidative phosphorylation, respiratory control, reversed electron transport, ATPase and pyrophosphatase activities. (Supported by Swedish Natural Sciences Research Council). ▪ ▪ ▪ Department of Botany, King's College, London and Botanical Institute, University of Stockholm, Sweden.