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Methods used for protein extraction

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Abstract

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Flavin, M. and C. Slaughter. Methods "red for protein extraction. in recent years we have extracted Neurospora by hand grinding with alumina, which releases more protein than any other method we had tried, and we still "se it on a small scale. For large scale preparations we now "se a "laboratory homogenizer" manufactured by the Manton-Gaulin Company of Everett, Mass. This press, which occupies little floor space although it weighs 450 pounds, extracts 3 times more protein than alumina does and is a great deal more agreeable to "se. A kilogram of freshly harvested mycelium is dispersed as well as possible in a large blendor into an equal weight of buffer. This chilled suspension can be passed through the press in 3 minutes. On a larger scale, the procedure would probably have to be interrupted, since the extract is coming out at 40°C after 3 minutes. If the press jams it takes 2 hours to dismantle and clean it. We have hod no problem with this since we took to stationing a second man at the hand wheel, to release it the instant the pressure rises at-we 8,000 pounds/inch^2.

We hove always ultracentrifuged Neurospora extracts prior to fractionation. We originally started this because of poor results with acetone fractionation when centrifugation was omitted: a gelatinous precipitate formed at low acetone concentrations ond adsorbed some soluble protein. This procedure seems to be particularly necessary with extracts of starved cells. These are grown with limiting sulfur (Flavin 1965 Biochem. Biophys. Res. Commun. 20:652); the cell mass is 35% and the extractable protein 15% of normal. The extracts ore run for 4 hours in the Spinco 21 rotor. Besides the pellet and the fat pellicle, it is essential to discard about the top 15% of solution, even though no refractile difference can be seen. Otherwise, sooner or later the protein will float when a salt fraction is centrifuged, or a viscous solution will be obtained which can't be passed through sephadex. • • • Enzyme Section, National Heart Institute, Bethesda, Maryland.