Kapoor, M. and D. Bray. Catabolite effects on some enzymes of Neurospora

During on experiment designed to study the effect of growth conditions on the activity and synthesis of glutamine synthetase, several interesting observations were made. Neurospora crassa pe (Y 8743m) (FGSC #37) was used as a source of enzymes in this study. All cultures were prepared in Vogel's minimal medium with sucrose or glucose as a carbon source, and mycelial powders were obtained as described in the communication on phosphofructokinase in this issue of the Neurospora Newsletter. The cultures were grown for 30 hours at 28°C. There was no autolysis in cultures with low concentration of sucrose. At the end of 30 hours not all of the sugar in the medium was exhausted, a very small amount remaining. Crude extracts were prepared by extracting at 3°C lyophilized mycelium powder in 0.05 M phosphate buffer ($5 \times 10^{-4}$ M in EDTA and $10^{-4}$ M in ß-mercaptoethanol) pH 7.5, for 30 minutes, straining the mixture through four layers of cheesecloth and centrifuging the supernatant at 27,000 x g for 15 minutes. The residue was discorded and the supernatant was used without further purification.

Table 1. Effect of sucrose on some enzymes of Neurospora.

| Sucrose concentration | Specific activity ( OD/mg protein) GDH-D | GDH -T | GluN-S'ase | PK |
|---|---|---|---|---|
| 0.1% | 0.75 | 0.05 | 0.005 | 0.12 |
| | 0.75 | 0.05 | 0.000 | 0. 12 |
| 0.5% | 0.56 | 0.37 | 0. 18 | 1.30 |
| | 0.53 | 0.38 | 0. 17 | 1.40 |
| 1 0 % | 0.45 | 0.70 | 0.32 | 2.00 |
| | 0.43 | 0.70 | 0.32 | 2.20 |
| 1.5% | 0.23 | 0.81 | 0.41 | 2.1 |
| | 0.24 | 0.79 | 0.41 | 2. 1 |
| 2.0% | 0. 15 | 0. 86 | 0.42 | 1.75 |
| | C. 15 | 0.86 | 0.42 | 2.00 |
| 2.5% | 0.12 | 0.98 | 0.45 | 2.59 |
| | 0. 19 | 0.98 | 0.44 | 2.30 |

Activities of glutamine synthetase (GluN-S'ase), NAD-specific glutamate dehydrogenase (GDH-D), NADP-specific glutamate dehydrogenase (GDH -T) and pyruvate kinase (PK ) were determined in extracts of mycelia obtained from cultures grown in different concentrations of sucrose. Arrays of the activities of the two glutamate dehydrogenases were performed with a Gilford model 2000 recording spectrophotometer by following the initial decrease in OD at 340 mμ accompanying the reductive amination of α-ketoglutarate in the presence of ammonia and reduced NAD or reduced NADP (K apoor and Smith 1968 Can. J. Microbiol. 14:609). GluN-S'ase was assayed by measuring the formation of γ-glutamyl hydroxamate from L-glutamate and hydroxylamine in the presence of ATP (K apoor and Bray 1968 Biochemistry 7:3583). PK was measured by following the decrease in OD at 340 mμ in the following reaction mixture at 25°C: Tris-H Cl, pH 8.0, 100 μmoles; $MgCl_2$ IO μmoles; ADP 1 μmole; reduced NAD 0.14 μmole; PEP 0.6 μmole; LDH (Sigma) 100 μg and enzyme preporation in a total volume of 3 ml.

Table I shows the specific activities of there enzymes in crude extracts of Neurospora mycelium grown at concentrations varying from 0. 1% to 2.5%. Glutamine synthetase is not repressed by sucrose and neither is pyruvate kinase; both there enzymes show on increase in specific activity in the presence of sucrose up to 1.5% but no further increase was noted at 2% and 2.5% sucrose. A study of the response of the two GDH's towards sucrose in the growth medium revealed a dramatic feature of regulation of GDH-T and GDH-D. Whereas GDH-D is subject to catabolite repression by sucrose and glucose, GDH-T is induced under the same conditions, thus demonstrating a reciprocal relationship between these two enzymes. It is already known that in the presence of glutamate or ammonia in the medium GDH-D is induced with a simultaneous repression

of GDH-T (Sanwal and Lata 1962 Arch. Biochem. Biophys. 98:420). It has been suggested that GDH-D is primarily a catabolic enzyme and that GDH-T serves an anabolic function in the cell. Our studier are in agreement with this suggestion in so for as it is GDH-D alone that is subject to catabolite repression and that GDH-T is induced under the same conditions. - - - Deportment of Biology, University of Calgary, Calgary, Alberta, Canada.