Preparative polyacrylamide disc electrophoresis (Davis, 1964; Ornstein, 1964) has been added as a final step in the purification to give an electrophoretically homogeneous preparation. The electrophoresis is carried out in an acrylamide gel with a high concentration of triethanolamine (0.2M) as both polymerization catalyst and cationic buffer component. The anionic buffer components are phosphate and diethylbarbituric acid, which give a running pH of 8.5.

Removal of the dithiothreitol from the enzyme preparations by dialysis renders the enzyme sensitive to inhibition by thymidine triphosphate. The molecular weight of the enzyme has been studied by Agarose gel filtration to give a value of 37 000 for both the crude and the purified enzymes, in both the presence and the absence of dithiothreitol.

The kinetics of the enzyme have been studied in the presence and absence of dithiothreitol. With deoxycytidine as substrate the enzyme shows no change in K_m (0.15mm) or V_{max} , whether in the S-form in the absence of dithiothreitol (thymidine triphosphate-sensitive), or in the D-form in the presence of dithiothreitol (desensitized to thymidine triphosphate). Nor is there evidence for any co-operativity with respect to substrate under these conditions. When thymidine triphosphate is added to the S-form the resulting inhibition is of a complex competitive type, with a parabolic relationship between K_m and thymidine triphosphate concentration. The Hill plot (Monod, Changeux & Jacob, 1963) for this inhibitor has a slope of 2 and a plot by the method of Dixon (1953) is non-linear. This seems to indicate co-operativity in the binding of thymidine triphosphate but none in the binding of the substrate.

Nucleotide inhibition patterns suggest that cytidine aminohydrolase is a low-molecular-weight enzyme with a single substrate site and separate interacting inhibitor sites.

N.V.McF. and M.S. are grateful to the Department of Education, Irish Republic, for grants. Financial support for this work from the Irish Cancer Society is also gratefully acknowledged.

Davis, B. J. (1964). *Ann. N.Y. Acad. Sci.* **121**, 404.

Dixon, M. (1953). *Biochem. J.* **55**, 170.

Monod, J., Changeux, J. P. & Jacob, F. (1963). *J. molec. Biol.* **6**, 306.

Ornstein, L. (1964). *Ann. N.Y. Acad. Sci.* **121**, 321.

Wisdom, G. B. & Orsi, B. A. (1967). *Biochem. J.* **104**, 7P.

Wisdom, G. B. & Orsi, B. A. (1969). *Europ. J. Biochem.* **7**, 223.

Melanin Production in *Aspergillus nidulans*

By S. J. PIRT and B. I. ROWLEY. [Department of Microbiology, Queen Elizabeth College (University of London), London W.8]

Little is known of the structure and function of melanins, though they are ubiquitous polymers in the plant, animal and protist kingdoms. After the characterization of melanin production in *Aspergillus nidulans* by Bull & Faulkner (1965), we undertook a study of the process, since it offered a promising system for the application of modern culture techniques and genetical methods to investigate the biogenesis and nature of melanins. In submerged batch culture on glucose-nitrate-inorganic salts medium, pH 6.8, the organism grew in the unpigmented form, and then over a period of many hours, after growth had ceased, the dark brown melanin accumulated in the mycelium. By application of the chemostat continuous-culture technique, it was established that melanin production is immediately induced when growth is glucose-limited over the range of specific growth rates up to at least 0.07 hr.⁻¹ (doubling time 9.9 hr.). The specific rate of melanin production in the chemostat increased considerably when the growth rate was decreased. Electron microscopy showed that melanin is laid down in a microfibrillar form on the exterior of the fungal hyphae. Some melanin was present in the medium in soluble form.

By application of automatic control (MacLennan & Pirt, 1966) it was found that melanin production was dependent on dissolved oxygen tension. Mycelial melanin production was maximal when the dissolved oxygen tension exceeded 30mm.Hg. At lower tension, the mycelial melanin production decreased in a manner dependent on the previous history of the culture, although mycelial growth (with specific growth rate 0.04 hr.⁻¹) was unaffected. The production of the soluble melanin rose sharply severalfold above the basal level when the dissolved oxygen tension was decreased below 30mm.Hg, reached a peak when the tension was about 18mm.Hg, then sharply fell at lower tension.

Methods for the extraction and assay of melanin have been developed. Chemical analysis indicates that *Aspergillus nidulans* melanin is a co-polymer of dihydroxyphenylalanine residues and non-nitrogenous monomers. The absorption spectra of the mycelial and soluble forms of *A. nidulans* were, in the visible range, similar to those of melanin extracted from *Sepia* and that synthesized by aeration of dihydroxyphenylalanine.

This work was supported by a grant from the Science Research Council.

- Bull, A. T. & Faulkner, B. M. (1965). *J. gen. Microbiol.* **41**, iv.
 MacLennan, D. G. & Pirt, S. J. (1966). *J. gen. Microbiol.* **45**, 289.

Phenol Oxidases Produced by Mutant and Wild-Type Strains of *Aspergillus nidulans*

By S. D. MARTINELLI and B. W. BAINBRIDGE (introduced by S. J. PIRT). [Department of Microbiology, Queen Elizabeth College (University of London), London W.8]

A survey has been made of the occurrence of phenol oxidase in wild-type and mutant strains of *Aspergillus nidulans*. Certain phenol oxidases are thought to be involved in melanin synthesis, and therefore mutant strains have been isolated with abnormal melanin synthesis.

In strain BWB224, our standard strain, five bands of dihydroxyphenylalanine oxidase activity or diphenol oxidase activity can be detected by polyacrylamide disc electrophoresis. Six bands have been detected in a mutant strain. Several other workers have described heterogeneity in phenol oxidases in fungi (Carter & Bull, 1968; Esser, 1963; Horowitz *et al.* 1961; Jolley, 1966).

Changes in the phenol oxidase patterns throughout growth have been followed in batch culture. During active growth, the number of phenol oxidase types increases, reaching a maximum in the stationary phase. During autolysis, when melanin is being deposited in the walls, no new phenol oxidases appear.

Differential centrifugation has shown that specific phenol oxidase bands are associated with different particulate fractions, but all phenol oxidases can be seen in the soluble fraction. The cellular distributions of these enzymes at different stages in the life cycle have also been compared. Although the total number of phenol oxidase types present in the lag, exponential, stationary and autolytic phases differs, the type of distribution is similar. Only one or two phenol oxidases are present in low-speed fractions (up to 12000g). All but one type occurs in the high-speed fraction (100000g), whereas all types can be detected in the final supernatant.

After incubation at 50° for 1 min., one phenol oxidase is inactivated in samples from each stage in the growth cycle. Further characterization of the *A. nidulans* phenol oxidases by substrate and inhibitor specificities should indicate their inter-relationships.

Parameters of growth, morphology, phenol oxidases and melanin pigmentation have been compared in a variety of wild-type isolates and

mutants. Fewer phenol oxidase types are found in a highly melanized mutant, whereas up to six bands appear in a mutant devoid of melanin. The wild-types show a range of phenol oxidase patterns, from which it may be possible to classify them into groups.

Both mutants have been assigned to linkage group VII, and an attempt to map them further is in progress.

This work was supported by a grant from the Science Research Council.

- Carter, B. L. A. & Bull, A. T. (1967). *J. gen. Microbiol.* **48**, iii.
 Esser, K. (1963). *Arch. für Mikrobiol.* **46**, 217.
 Horowitz, N. H., Fling, M., McLeod, H. & Sueoka, N. (1961). *Genetics*, **46**, 1015.
 Jolley, R. L., jun. (1966). *Advances in the Biology of Skin*, vol. 3: *The Pigmentary System*, p. 269. Oxford: Pergamon Press.

The Effect of 3-Deoxy-3-fluoro-D-glucose, a Non-metabolizable Sugar, on the Metabolism of *Saccharomyces cerevisiae*

By R. J. MILES and S. J. PIRT. [Department of Microbiology, Queen Elizabeth College (University of London), London W.8]

On the basis of extent of oxygen uptake, carbon dioxide production and the activity of 3-FG* in various enzyme systems, Brunt & Taylor (1967) concluded that 3-FG was metabolized by yeast. This conclusion is in disagreement with our findings.

We find that: (1) 3-FG is not a growth substrate for *Saccharomyces cerevisiae* (strain 229, National Collection of Yeast Cultures); (2) when present in equimolar amount with glucose, 3-FG does not affect the growth of the yeast in any way (lag phase, doubling time or growth yield); (3) 3-FG does not disappear from the medium. We developed an assay for 3-FG based on its oxidation by glucose oxidase, the rate of which was directly proportional to the concentration of 3-FG. The rate of oxidation was much less (0.3%) than the initial rate for glucose under the same conditions of assay.

When 3-FG was added to yeast cells (from glucose-limited cultures) in Warburg respirometers the respiration rate increased up to sixfold. However, again 3-FG did not disappear to a significant extent from the flasks. The source of the substrate for respiration was investigated with yeast cells that had ¹⁴C-labelled carbon (the cells were grown on uniformly labelled glucose). By this means it was found that the carbon dioxide evolved when 3-FG was added to a resting yeast suspension was exclusively of cellular origin and not from 3-FG.

* Abbreviation: 3-FG, 3-deoxy-3-fluoro-D-glucose.