Chem. **166**:397, 1946), which requires biotin, pantothenate (or β-alanine), and NA for optimal growth; thiamine is only stimulatory. Pantothenate (or β-alanine) stimulates but is not essential for the growth of CCRF no. 60705. *m*-Inositol (2.0 μg/ml) and pyridoxine (2.0 μg/ml) are only stimulatory, as was the case with *T. cremoris* ATCC no. 2512. Other vitamins, purines, pyrimidines, etc., had no significant effect upon the growth of CCRF no. 60705.

Comparative studies with nonrequiring strains of the same species indicate that the NA requirement of CCRF no. 60705 probably results from genetic blocks similar to those described in Neurospora (Mitchell and Nye, Proc. Natl. Acad. Sci. U. S. **34**:1, 1948; Bonner and Beadle, Arch. Biochem. **11**:314, 1946), as judged by inability to utilize L-tryptophan, L-kynurenine, or 3-hydroxyanthranilic acid as precursors of NA.

Bio-assay and mechanism of action studies with a series of 1,2-dihydro-*s*-triazines (Foley, Modest, Cataldo, and Riley, Biochem. Pharmacol., **3**:18, 1959) with CCRF no. 60705 in inorganic media, supplemented with NA, biotin, and thiamine, indicates good agreement with data obtained in the usual *Lactobacillus arabinosus* and NA assay system.

These studies were supported in part by research grants C-2309(C) and CY-3335 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

OXIDATION OF METADIHYDROXYPHENOLS BY ENZYMES OF *PIRICULARIA ORYZAE*

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Received for publication April 17, 1961

Piricularia oryzae possesses an enzyme that catalyzes the oxidation of 1,3-dihydroxyphenols (Neufeld, Latterell, and Weintraub, Arch. Biochem. Biophys. **76**:317, 1958). This communication presents findings regarding the mechanism of oxidation involved with this metapolyphenoloxidase, and argues against the hypothesis that 2,2′-dihydroxy-4,4′-diphenoquinone or related compounds are formed. These have been reported as intermediates or as end products of the nonenzymatic oxidation of resorcinol (Heinrich, Setz. phys. med. soc. Erlangen **71**:199, 1939; Valyashko and Shcherbak, J. Gen. Chem. (U. S. S. R.) **8**:1641, 1938), or they might be postulated from simple stoichiometric considerations when this compound is used as a substrate.

Enzymes were prepared and oxidation was measured as described by Neufeld et al. (Arch. Biochem. Biophys. **76**: 317, 1958). When oxygen uptake ceased, the reaction mixture was acidified and lyophilized, and the residue was extracted with methanol and chromatographed (*n*-butanol-pyridine-water, 3:2:1.5). Spots were visualized with ultraviolet light and their absorption spectra were determined. The resorcinol reaction gave fluorescent spots with the following R_F values: 0.19, 0.27, 0.62, 0.91; orcinol gave spots 0.10, 0.40, 0.56, 0.89. The material of spot R_F 0.27 was a hygroscopic glass, carbonized without melting, and exhibited absorption maxima at 250, 256, 262, and 425 mμ. Reducing agents caused the 425 mμ peak to shift to 386 mμ. In the infrared spectrum, strong quinoid, hydroxyl, and ether bands were observed. Spectra of the remaining resorcinol- and orcinol-derived spots also exhibited the ultraviolet band triad, and materials eluted from these spots were reducible.

Two possible precursor phenols were incubated with the enzyme. 2,4,2′,4′-Tetrahydroxybiphenyl and 2,9-dihydroxydiphenylene oxide, which theoretically could yield 2,2′-dihydroxy-4,4′-diphenoquinone or (when followed by loss of a molecule of water), could yield the corresponding 2,7-dioxodiphenylene oxide. Slow polymerization was observed with little oxygen uptake. When the tetrahydroxy compound was oxidized with silver oxide or with oxygen in alkaline solution containing cupric ion, different polymers were obtained in either system that were not identical with the products from the corresponding enzyme-catalyzed reaction. The diphenylene oxide failed to oxidize in alkali, but gave the expected 2,7-dioxodiphenylene oxide

with silver oxide. Resorcinol, when oxidized in alkaline solution, yielded a product identical with the substance from spot R_F 0.27, and the expected diphenoquinone.

Another phenol, 2,4,5-tribromoresorcinol, polymerized rapidly without oxygen consumption and without liberation of bromide ion when subjected to the action of the enzyme.

Oxidation mechanisms for phenolases such as those proposed by Nelson and Dawson (J. Am. Chem. Soc. **61**:245, 1938), where a phenol is oxidized directly by oxygen, cannot explain the lack of oxygen utilization in the tribromoresorcinol oxidation. Also not applicable are mechanisms postulating reduction of the cupric ion moiety in such enzymes concomitant with the formation of a quinone and followed by subsequent regeneration of the cupric state via oxidation with elemental oxygen (Kubowitz, Biochem. Z. **292**:221, 1937). The rapidity of the reaction, and the polymeric nature of the products with this as well as with other phenols, indicates the formation of an intermediate similar to a free radical. Failure to utilize oxygen in these cases suggests the creation of conditions such that a chain-sustaining polymerization occurs without further participation of enzyme or oxygen.

In those reactions where oxygen was consumed, the diversity of products could arise by utilization of primary oxidation or coupling products as substrates for accompanying phenoloxidases, as Neufeld et al. have shown their enzyme preparation to be heterogeneous.

The author wishes to acknowledge the collaboration of H. A. Neufeld.

NEW INDOLE TEST REQUIRING ONLY SIMPLE REAGENTS

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Received for publication April 20, 1961

Although Kovacs' method is now commonly used in testing for indole production by *Enterobacteriaceae*, it requires the use of *p*-dimethylaminobenzaldehyde. This compound is not always easy to obtain in some areas of the world and it is unstable in solution, necessitating storage in the cold.

To develop an indole test which can be carried out in any laboratory with common reagents, the combined action of formaldehyde, sulfuric acid, and potassium dichromate was utilized as follows.

Two separate solutions are necessary:

Solution I. Formalin (about 37% formaldehyde), 1 part; concentrated sulfuric acid, 2 parts. Mix cautiously in a pyrex Erlenmeyer flask, pouring the sulfuric acid very slowly into the formaldehyde. This mixture keeps indefinitely.

Solution II. Potassium dichromate, 0.5% in distilled water.

Use. For each milliliter of 24- to 48-hr bacterial broth culture to be tested, add two drops (about 0.1 ml) of solution I. Invert the stoppered tube to mix the contents completely. Then add, for each milliliter of culture, one drop of the solution II and mix. A distinctly pink hue appears immediately whenever indole is present, in contrast to an accentuated yellow color seen in the absence of indole.

This reaction has been checked by comparison with Kovacs' reaction in more than two thousand (2,234) tests on different strains of *Enterobacteriaceae*; without exception both tests were coincident. It appears that this test could be submitted advantageously as a method for demonstrating indole production when the reagents for the Kovacs' method, especially *p*-dimethylaminobenzaldehyde, are not available.