NOTES

MALATE SYNTHETASE AND ISOCITRITASE IN *TETRAHYMENA PYRIFORMIS*

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The two key enzymes of the glyoxylate cycle (Kornberg and Krebs, Nature, **179,** 988, 1957), malate synthetase discovered in our laboratories (Wong and Ajl, J. Am. Chem. Soc., **78,** 3230, 1956) and isocitritase, originally described by Campbell et al. (Biochim. et Biophys. Acta, **11,** 594, 1953) and subsequently by Olson (Nature, **194,** 695, 1954), Saz (Biochem. J., London, **58,** xx, 1954), Saz and Hillary (Biochem. J., London, **62,** 563, 1956), and Smith and Gunsalus (J. Am. Chem. Soc., **76,** 5002, 1954; Nature, **175,** 774, 1955; J. Biol. Chem., **229,** 305, 1957) have been found to be widely distributed in nature. These two enzymes have been reported to occur in numerous bacterial species, yeasts, molds, and certain plant tissues (for listing, *see* Kornberg, Ann. Rev. Microbiol., **13,** 49, 1959). It was of interest, therefore, to determine if these enzymes also occur in the protozoan, Tetrahymena; Hogg (Federation Proc., **18,** 247, 1959) mentions them in reference to gluconeogenesis in the E strain of this organism.

TABLE 1

Specific activities of malate synthetase and isocitritase in cell-free extracts of Tetrahymena pyriformis

Enzyme	Specific Activity
	μmole:min:mg protein
Malate synthetase	0.032
Isocitritase	0.002

Tetrahymena pyriformis was used; this strain has been reported by Seaman (J. Biol. Chem., **228,** 149, 1957) to contain an enzyme which reversibly cleaves succinate to two C_2-units. Cells were grown with vigorous aeration at 25 C for 4 days in a medium containing 1.0 per cent proteose peptone no. 3 (Difco) and 0.1 per cent yeast extract (Difco). The cells were harvested and resuspended in 0.05 M phosphate buffer at pH 7.4

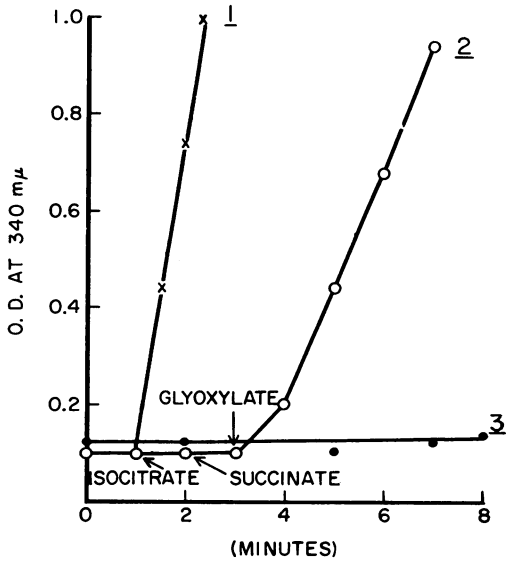

Figure 1. Isocitric dehydrogenase activity and the reversibility of the isocitritase reaction. Total volume of reactants, 3.0 ml, in Beckman quartz cells of 1 cm light path. Cuvettes contained: 50 μmoles tris(hydroxymethyl)aminomethane buffer (pH 8.0); 10 μmoles $MgCl_2$; 0.2 mg triphosphopyridine nucleotide; 0.3 ml of extract of *Tetrahymena pyriformis*; and water. *Curve 1,* 5 μmoles of isocitrate were added. *Curve 2,* 5 μmoles of succinate were added followed by the addition of 10 μmoles of glyoxylate. *Curve 3,* 10 μmoles of glyoxylate added; no succinate. Optical density was measured at 340 mμ.

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containing 10 μmoles of neutralized cysteine hydrochloride per ml. Cell-free extracts were prepared using a sonic oscillator followed by centrifugation at 11,000 rev/min for 20 min at 4 C to remove cell debris.

Malate synthetase and isocitritase activities were determined spectrophotometrically by the method described elsewhere (Reeves and Ajl, J. Bacteriol., **79,** 341, 1960). Protein concentrations were estimated by the method of Warburg and Christian (Biochem. Z., **310,** 384, 1941).

The data presented in table 1 show the specific activities of malate synthetase and isocitritase in *T. pyriformis*. It can be noted that the specific activity of malate synthetase is nearly 10-fold that of isocitritase. This finding is in accord with our earlier data concerning the specific activities of these two enzymes in *Escherichia coli* (Reeves and Ajl, J. Bacteriol., **79,** 341, 1960). Figure 1 presents the details of the isocitritase determination. It is clear that this enzyme and not isocitric dehydrogenase, determined the rate of the coupled reaction of curve 2.

Although the role of both malate synthetase and isocitritase is well established for the aerobic growth of bacteria on such C_2-compounds as acetate, their function in Tetrahymena grown in complex medium remains to be elucidated.

PREPARATION OF STABLE *LISTERIA MONOCYTOGENES* O ANTIGEN

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It is well known that boiled suspensions of *Listeria monocytogenes* generally used for the detection of O antibodies in human and animal sera are quite unstable. Paterson (J. Pathol. Bacteriol., **51,** 427, 1940) was not able to test some strains because of failure in obtaining stable O suspensions. Similar observations can be found also in the recent literature (Hood, Am. J. Clin. Pathol., **28,** 18, 1957). Seeliger (*Listeriose*, 2nd ed., J. A. Barth, Leipzig, 1958) emphasized the use of strongly buffered glucose agar for cultivation, buffered saline for harvesting the bacterial growth, and the treating of the boiled suspensions in the ultrasonator for the dispersion of clumps observed after heating.

Preliminary experiments in our laboratory indicated that we regularly obtained unstable suspensions when employing different media for cultivation and when using unbuffered or buffered saline solutions for washing off the growth. On the basis of these results it was decided to try the cultivation of Listeria on a cellophane layer which excluded the possibility of direct contact between the culture medium and the bacterial cell surface, and to use distilled water instead of saline for harvesting the bacteria.

Blood agar plates, with 5 per cent human blood, covered with a sterile cellophane membrane, and dried in the incubator at 37 C, were inoculated with different Listeria strains. Plates were incubated at 37 C for 48 hr, and the bacteria were washed off with sterile distilled water, pH 7.4. After harvesting, the suspensions were boiled for 2 hr and stored in the refrigerator for 6 months. A number of suspensions were stored in saline solutions: 0.2, 0.4, and 0.85 per cent, pH 7.4. Some lots were preserved by 0.01 per cent merthiolate.

Fifty Listeria strains were tested by the above method. They were obtained from human or animal infections from various parts of the world, among them some recently isolated strains from Hungary. The strains represented all Listeria serotypes and subtypes hitherto recognized. All strains developed well on blood agar covered with cellophane membrane and yielded stable suspensions in every case after harvesting the growth by distilled water. The stability of the suspensions was not altered by boiling for 2 hr, and the boiled suspensions showed no tendency of clumping during the whole period of storage, whether the suspending fluid was distilled water or 0.2 to 0.85 per cent saline.

Agglutination experiments showed that these O antigens reacted easily and specifically with animal and human sera containing Listeria O antibodies. Similar results were obtained with hyperimmune rabbit sera produced by injecting boiled Listeria suspensions of serotypes 1, 2, 3, 4a, 4b, 4c, 4d, and 4e. The O antigens were