hypotaurocholate, whereas addition of hypotaurine had no significant effect on the taurocholate formation. Our experiments therefore show that rat liver microsomes can conjugate hypotaurine with cholic acid, but that taurine is preferred as substrate in the reaction. Since rat liver is known to contain great amounts of free taurine⁵, the conjugation with hypotaurine can only be of limited physiological importance in the rat.

Whether the conversion of hypotaurine to taurine is enzymatic or spontaneous in our system is not known.

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Formation of Itaconic Acid from the Krebs Cycle Tricarboxylic Acids by Extracts of *Aspergillus terreus*

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Evidence has been presented that citric acid^{1,2} and *cis*-aconitic acid³ are intermediates and close precursors to itaconic acid in the formation of itaconic acid from sugars by *Aspergillus terreus*.

Cell-free extracts of *A. terreus* can be prepared by crushing rapidly frozen mycelium in the Hughes' bacterial press and extracting the paste with water. Such extracts, when prepared from itaconic acid producing mycelia (*i. e.*, mycelia grown under acid conditions⁴), will catalyze a conversion of citric, *cis*-aconitic and isocitric acids to itaconic acid and carbon dioxide. *cis*-Aconitic acid is by far the most readily converted; citric and isocitric acids were in some experiments converted to itaconic acid only to a negligible extent. The optimum pH is about 5.7. Molecular oxygen does not affect the conversions. Extracts prepared from mycelia grown under neutral con-

ditions (*i. e.*, mycelia not producing itaconic acid from sugars⁴), will not catalyze a conversion of the aforementioned tricarboxylic acids to itaconic acid. This finding is in agreement with the previously presented hypothesis that the itaconic acid forming enzyme system is dependent upon acid conditions for its formation⁴.

Active extracts decarboxylate 1 mole of *cis*-aconitic acid to give close to 1 mole of carbon dioxide and 1 mole of itaconic acid. Correspondingly, the yield of carbon dioxide from citric acid is close to 1 mole per mole of citric acid converted, whereas the yield of itaconic acid is usually much lower, *viz.* 0.3—0.6 moles per mole of citric acid converted.

Synthetic monofluorocitrate in a concn. of 10⁻⁴ M causes an appreciable inhibition in the rate of itaconic acid production from citric acid and a slight inhibition in the rate of itaconic acid production from isocitric acid. 10⁻⁴ M fluorocitrate does not inhibit the rate or lower the yield of itaconic acid formation from *cis*-aconitic acid; there is rather a slight stimulation. Aconitase has been shown to be present in the extract. Assuming the aconitase to be fluorocitrate sensitive⁵, the results would indicate that itaconic acid is formed by decarboxylation of *cis*-aconitic acid.

The reversibility of the decarboxylating system has been tested by incubating itaconic acid producing extracts in the presence of *cis*-aconitic acid and ¹⁴CO₂ at pH 5.3. Itaconic acid and residual *cis*-aconitic acid were isolated and tested for radioactivity. Both compounds were found to be non-radioactive. The lack of radioactivity in the *cis*-aconitic acid does not necessarily imply that the conversion is irreversible; it might equally well indicate an extreme slowness in the mixing of the *cis*-aconitic acid supplied and its biologically activated counterpart⁶.

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