NOTES

EVIDENCE FOR A TRICARBOXYLIC ACID CYCLE IN CORYNEBACTERIUM CREATINOVORANS

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In their studies of oxidations by acetate-grown Corynebacterium creatinovorans, Barron and coworkers (Arch. Biochem., 29, 130, 1950) were unable to demonstrate the formation or utilization of sodium citrate by this organism during acetate oxidation. On the basis of this study and inhibitor data, these workers concluded that a tricarboxylic acid cycle does not exist in this organism and further postulated that acetate oxidation by this organism must occur by means of an alternate pathway, e.g., a dicarboxylic acid cycle. Because of the significance of this study to our knowledge of terminal respiration, a re-examination of acetate oxidation by this organism was undertaken. The present note presents manometric and spectrophotometric evidence to show that the tricarboxylic acid cycle does function in C. creatinovorans.

In our laboratory, as in the studies of Barron and coworkers, whole cells were found to oxidize the various intermediates of the tricarboxylic acid cycle, with the exception of the six-carbon intermediates, such as sodium citrate. However, it could be demonstrated spectrophotometrically, by following the reduction of triphosphopyridine nucleotide (Adler, E., et al., Biochem. J., 33, 1028, 1939), that sodium citrate and sodium isocitrate were readily oxidized by sonic preparations of C. creatinovorans (figure 1). This indicates that the absence of citrate oxidation by whole cells was due to impermeability.

Further, during the oxidation of sodium malate or sodium pyruvate in the presence of sodium fluoroacetate, citrate could be shown to accumulate, whereas in the absence of fluoroacetate no significant levels of citrate accumulated. The values in table 1 represent the average of six experiments; thus while the amounts of citrate trapped are small they would appear to represent significant amounts.

When sodium acetate is the substrate, little citrate accumulates in the presence of sodium fluoroacetate. Results similar to these were obtained by Altenbern and Housewright (Arch. Biochem., 36, 345, 1952) with Brucella abortus, which has led the latter workers to postulate that fluoroacetate was inhibiting the formation of a 4-carbon unit from acetate. However, in view of the data (table 1), which show that when acetate is oxidized in the presence of fluoroacetate the amount of citrate trapped is approximately the same as the endogenous value, either (1) that in the presence of relatively high concentrations of acetate the inhibitory action of fluoroacetate is overcome or (2) that an alternate fluoroacetate-insensitive pathway of acetate oxidation exists.

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Figure 1. The oxidation of citric and isocitric acids by sonic-extracts.
Protocol: Veronal buffer, pH 7.0, 1.0 ml; 1 μM MnCl₂; 0.2 ml enzyme; 0.3 μM triphosphopyridine nucleotide; 0.5 ml sodium citrate or isocitrate; total volume, 3.0 ml.

TABLE 1
The accumulation of citric acid during oxidation by Corynebacterium creatinovorans in the presence of sodium fluoroacetate*

<table>
<thead>
<tr>
<th>SUBSTRATE</th>
<th>SODIUM FLUOROACETATE</th>
<th>μM OXYGEN UPTAKE</th>
<th>μM CITRATE ACCUMULATED†</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>none</td>
<td>10.0</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>5 × 10⁻⁷ M</td>
<td>9.1</td>
<td>0.64</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>none</td>
<td>63.0</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>5 × 10⁻⁷ M</td>
<td>15.0</td>
<td>0.60</td>
</tr>
<tr>
<td>Sodium malate</td>
<td>none</td>
<td>56.0</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>5 × 10⁻⁷ M</td>
<td>20.2</td>
<td>1.03</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>none</td>
<td>61.0</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>5 × 10⁻⁷ M</td>
<td>17.0</td>
<td>1.30</td>
</tr>
</tbody>
</table>

* Obtained from Monsanto Chemical Company, St. Louis, Missouri.
† Citrate was determined using a modification of the method of Taussky and Shorr (J. Biol. Chem., 181, 195, 1948).
The foregoing evidence would seem to indicate that a tricarboxylic acid cycle does exist in *C. creatinovorans*. However, the foregoing data do not rule out the possibility of the coexistence of an alternate terminal respiration cycle, e.g., a dicarboxylic acid cycle in this species.

EFFECTS OF SODIUM CHLORIDE ON THE DOSAGE-RESPONSE CURVE OF LACTOBACILLUS LEICHMANNII TO VITAMIN B₁₂

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The dosage-response curves of *Lactobacillus leichmannii* (American Type Culture Collection strains 4797 and 7830) have generally been found to be of logarithmic form (Brownlee and Lapedes, J. Bact., 62, 433, 1951; Hoffmann et al., J. Biol. Chem., 176, 1465, 1948). Also, the latter strain has been found to respond to considerably lower concentrations of vitamin B₁₂.

In the course of investigations with 15 mg NaCl per ml of basal assay medium, *L. leichmannii* (strain ATCC 7830) was found to give a straight-line response, arithmetically, within a concentration range of 0.4 to 1.6 mug B₁₂ per 10 ml after incubation for 22 to 24 hours, instead of the usual logarithmic curve between 0.01 and 0.1 mug after 14 to 16 hours. It was found that different potencies of vitamin solution resulted in varying slopes of the linear curves. Relative potencies were then calculated by means of regression coefficients (Finney, Quart. J. Pharm. Pharmacol., 18, 70, 1945).

The media employed were those described in the U.S.P. XIV, Third Supplement, based on studies by a designated group (Loy et al., J. Assoc. Offic. Agr. Chemists, 35, 161, 1952). Stock cultures of the organism were maintained in the enriched medium with transfers at 48 hr intervals and inocula prepared from 24 to 48 hr cultures. Incubation was at 37 C. To verify the straight-line nature of the arithmetic curve, ten replicate tubes were inoculated at increments of 0.2 mug from 0.6 to 1.6 mug inclusive. The respective average growth densities were as follows: 26.0, 32.8, 36.5, 44.1, 48.1, and 52.6. Statistical analysis of these data has shown that 67 per cent of all readings within the range may be expected to fall within one standard deviation of a straight line.

The accompanying table summarizes data and statistics on two B₁₂ concentrates, one (N) derived from a fermentation and the other (P) from liver. Mean results (\( \bar{x} \)) and standard deviations (s) are expressed in \( \mu g \) per ml. Using the standard deviation of differences in means (sₐ) as the criterion, the data were tested for significant differences in results for each sample by means of the equations

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(1) \quad s_\Delta = \sqrt{\frac{n_1 s_1^2 + n_2 s_2^2}{n_1 + n_2}} \quad (2) \quad k = \frac{\bar{x}_1 - \bar{x}_2}{s_\Delta}
\]