During the past few years scattered information has appeared which indicates that at least some bacteria probably possess a tricarboxylic acid cycle. Lockwood and Stodola found that Pseudomonas fluorescens formed considerable quantities of α-ketoglutaric acid when growing on a glucose medium under conditions of intense aeration (1). Ajl and Werkman found evidence for at least parts of the conventional cycle in Escherichia coli (2). However, Karlsson and Barker (3) were not able to demonstrate the presence of this system in Azotobacter agile, either by the technique of simultaneous adaptation or by radioactive tracers. In contradiction to this latter work Stern and Ochoa (4) were able to show that both A. agile and E. coli possess the enzyme necessary for the condensation of oxalacetate and “active acetate” to citrate. Since Karlsson and Barker did not report on the activity of the organism towards the C₄ members of the tricarboxylic acid cycle, it is not possible to decide whether the technique of simultaneous adaptation is applicable to the detection of the intermediates of the tricarboxylic acid cycle or whether A. agile possesses only fragments of the cycle.

The present study was undertaken in an effort to detect a tricarboxylic acid cycle in Pseudomonas aeruginosa and thereby to elucidate further the pathway of glucose oxidation in this organism.

**Methods**

*P. aeruginosa*, ATCC 9027, which is a typical strongly pigmenting strain, was used throughout. Conditions for the growth of the organisms and methods of treating the dried cell preparations were the same as those described earlier (5, 6). For experiments involving resting cells, the cultures were grown either on a glucose-ammonium phosphate medium or on an acetate-ammonium phosphate medium, depending on the adaptive pattern desired. Cells were harvested after 21 hours at 30°, washed in saline, and resuspended at a concentration of 0.30 mg. of bacterial nitrogen per ml. Each reaction vessel contained 0.5 ml. of the standardized suspen-

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sion. For studies with dried cells the preparations were accurately weighed and suspended in distilled water at a rate of 40 mg. per ml. Each reaction vessel contained 0.5 ml. of this suspension.

With the exception of cis-aconitic acid, all substrates were commercial preparations. The cis-aconitate was prepared from the trans isomer by the procedure of Malachowski and Maslowski (7). The DPN (diphosphopyridine nucleotide) used in the manometric work was the "75 per cent purity" product of the Schwarz Laboratories. The cytochrome c was the product of the Sigma Chemical Company. In preliminary work DPN and cytochrome c, kindly donated by Dr. P. K. Stumpf, were employed.

EXPERIMENTAL

P. aeruginosa appears to be very similar in its adaptive enzyme pattern to the strain of A. agile used by Karlsson and Barker (3). Resting cells of this strain of Pseudomonas harvested from an acetate medium were found to have no initial ability to attack citrate, cis-aconitate, isocitrate, α-ketoglutarate, succinate, or fumarate. However, they were active towards malate, oxalacetate, and pyruvate. In the case of malate the reaction could be termed adaptive, for the rate increased markedly after the first 15 minutes (Fig. 1). Interpreted in the light of the theory of simultaneous adaptation, this would mean that the oxidation of acetate does not involve citrate, cis-aconitate, isocitrate, α-ketoglutarate, succinate, or fumarate and therefore does not involve the tricarboxylic acid cycle. However, when dried cell preparations were produced from acetate-grown cells, it was found that they had the ability to oxidize all of the intermediates of the tricarboxylic acid cycle which were tested (Table I). These compounds included all members of the cycle with the exception of oxalosuccinic acid and thus point to the presence of a complete tricarboxylic acid cycle in this organism.

Apparently the cell membrane of P. aeruginosa was initially impermeable to the intermediates mentioned and the period of adaptation encountered with resting cells was the time necessary for the elaboration of the system for transferring the substrates across the membrane. It is apparent, therefore, that the technique of simultaneous adaptation cannot be relied on to identify a very high percentage of metabolic intermediates. This becomes particularly evident when one realizes that the vast majority of intermediate compounds may be phosphorylated and therefore will have difficulty in passing across a cell membrane.

Resting cells harvested from a glucose medium had no ability to attack citrate, isocitrate, cis-aconitate, or succinate immediately. However, activity was demonstrated with acetate, α-ketoglutarate, fumarate, malate, oxalacetate, and pyruvate (Fig. 2).
The oxidation of acetic, fumaric, malic, oxalacetic, pyruvic, succinic, α-ketoglutaric, isocitric, citric, and cis-aconitic acids by resting cells of *P. aeruginosa* harvested from an acetate medium. The theoretical oxygen uptake for complete oxidation of any substrate was 403 µl.

**Table I**

**Oxidation of Members of Tricarboxylic Acid Cycle by Dried Cells of *P. aeruginosa***

The Warburg cups contained 20 mg. of dried cells, 1.5 ml. of m/15 phosphate buffer, pH 7.2, 1 mg. of cytochrome c, 109 µg of DPN, 2 µM of MgSO₄·7H₂O, 2 µM of MnCl₂, and 5 µM of substrate. The values for O₂ uptake at 40 minutes are in every case almost exactly double the value recorded at 20 minutes. The endogenous respiration has been subtracted from each of the recorded values. The endogenous uptake of the acetate preparation was 45 µl., while that of the glucose preparation was 18 µl.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cells grown on acetate medium µl.</th>
<th>Cells grown on glucose medium µl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Citrate</td>
<td>34</td>
<td>26</td>
</tr>
<tr>
<td>cis-Aconitate</td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td>Isocitrate</td>
<td>50</td>
<td>39</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>115</td>
<td>28</td>
</tr>
<tr>
<td>Succinate</td>
<td>80</td>
<td>86</td>
</tr>
<tr>
<td>Fumarate</td>
<td>59</td>
<td>75</td>
</tr>
<tr>
<td>Malate</td>
<td>72</td>
<td>49</td>
</tr>
<tr>
<td>Oxalacetate</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>16</td>
<td>10</td>
</tr>
</tbody>
</table>
When cells were harvested from a glucose medium and dried over phosphoric anhydride to form a dried cell preparation, it was found that with the exception of acetate and oxalacetate the glucose-grown, dried cells had the ability to oxidize all of the intermediates tested (Table I). This confirms the results obtained with cells grown on acetate and indicates that glucose is oxidized by way of the tricarboxylic acid cycle.

It was found that even the dried cells prepared from a culture grown on acetate lost their ability to oxidize acetate within 48 hours of the time they were dried. It is evident therefore that the enzyme system for utilizing acetate is very readily destroyed. This would explain the negative results obtained with the dried cells prepared from the glucose culture.

**DISCUSSION**

The presence of the enzymes necessary for the oxidation of the members of the tricarboxylic acid cycle in cells of *P. aeruginosa* harvested from
either a glucose or an acetate medium points to the constitutive nature of the enzymes involved. Nevertheless, a period of adaptation was necessary before resting cells oxidized many of the intermediates. This finding is contrary to the conclusion of Sleeper, Tsuchida, and Stanier (8) that drying does not increase the range of compounds that can be attacked by bacterial cells. If the technique of simultaneous adaptation (9) is relied on for the detection of metabolic intermediates, a great many compounds will be erroneously eliminated. The use of simultaneous adaptation as a means of ruling out postulated metabolic intermediates presupposes either that the cell membrane of bacteria is permeable to all the intermediates formed during the degradation of substrate or that the intermediate metabolism of bacteria is entirely an extracellular process. Since neither of these suppositions is true, the limitations of the technique are obvious.

It is now known that P. aeruginosa 9027 oxidizes glucose by way of gluconic and 2-ketogluconic acids (6, 10) and that it does not possess an Embden-Meyerhof scheme of metabolism (10, 11). The point at which the pathway of glucose oxidation meets the tricarboxylic acid cycle is difficult to predict. However, since pyruvate has been established as an intermediate in glucose oxidation (12), it is likely that the oxidation of this intermediate to acetyl phosphate is of supreme importance as a means of oxidizing glucose by way of the Krebs tricarboxylic acid cycle. There is no evidence that this organism oxidizes glucose by way of a 3:3 split, and it is therefore possible that the cycle is also entered at the C4 level. If the scheme of glucose oxidation of this organism paralleled that proposed by Dickens and Glock (13) for animal tissue, it would be a stepwise procedure, going from C4 to C5, C4, and C2. However, the oxidation of glucose by the organism under study differs from that described by these workers at least at the C4 stage, for no esterification of phosphate occurs with P. aeruginosa in the steps from glucose to 2-ketogluconate (6).

**SUMMARY**

Resting cells of Pseudomonas aeruginosa, harvested from a growth medium containing acetate as the sole source of carbon, were found to have no ability to oxidize citrate, cis-aconitate, isocitrate, α-ketoglutarate, succinate, or fumarate without a period of adaptation, but were able to oxidize acetate, pyruvate, and malate immediately.

However, when these fresh resting cells were dried, it was found that they then had the ability to oxidize citrate, cis-aconitate, isocitrate, α-ketoglutarate, succinate, fumarate, malate, oxalacetate, pyruvate, and acetate immediately. Therefore the limiting factor in the immediate utilization of these intermediates by resting cells was the impermeability of the cell membranes.
Confirmatory data were obtained with cells grown on a glucose medium, indicating that this organism oxidizes glucose and acetate by way of the Krebs tricarboxylic acid cycle.

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