Microbial Metabolism of C₁ and C₂ Compounds

THE ROLE OF GLYOXYLATE, GLYCOLLATE AND ACETATE IN THE GROWTH OF PSEUDOMONAS AM1 ON ETHANOL AND ON C₁ COMPOUNDS

By PATRICIA M. DUNSTAN, C. ANTHONY and W. T. DRABBLE
Department of Physiology and Biochemistry, University of Southampton, Southampton S09 5NH, U.K.

(Received 15 December 1971)

Succinate (or a product of succinate metabolism) is a catabolite repressor of some enzymes of the serine pathway (hydroxypyruvate reductase, serine-glyoxylate aminotransferase and glycerate kinase) but not of methanol dehydrogenase nor methylamine dehydrogenase. A mutant (PCT64) of Pseudomonas AM1, which is unable to grow on C₁ compounds, lacks glycerate kinase, showing that this enzyme is essential for the operation of the serine pathway. Mutant PCT48, unable to convert acetate into glycollate, has lost the ability to grow both on C₁ compounds and on ethanol. The properties of a third mutant (PCT57) show that Pseudomonas AM1 contains enzymes catalysing the conversion of acetate into glyoxylate. Evidence is presented that hydroxypyruvate reductase is involved in the oxidation of glycollate to glyoxylate during growth on ethanol. A scheme is proposed for the conversion of ethanol and of C₁ compounds into glyoxylate in which acetate (or a derivative) and glycollate are intermediates.

Pseudomonas AM1 is a pink bacterium capable of growth on a variety of carbon sources including succinate, ethanol, oxalate and C₁ compounds. During the assimilation of C₁ compounds a C₁ unit condenses with glycine to give serine, which is then converted into glycolytic intermediates (Large et al., 1961; Large & Quayle, 1963). To maintain the operation of this serine pathway, glyoxylate, the precursor of glycine (Harder & Quayle, 1971b), must be synthesized from C₁ units. The intermediates involved in this part of the pathway for the assimilation of C₁ compounds are unknown. In Pseudomonas AM1 ethanol is not assimilated by way of the glyoxylate bypass but probably by a novel route involving acetate, glycollate and glyoxylate as intermediates (Dunstan et al., 1972). Results of work with mutants of Pseudomonas AM1 described in the present paper indicated that, during growth on C₁ compounds, glyoxylate is formed from C₁ units by way of a similar route involving acetate and glycollate as intermediates. Preliminary reports of this work have been published (Anthony et al., 1971a,b).

Experimental

Chemicals

All chemicals were obtained from British Drug Houses Ltd., Poole, Dorset, U.K., except the following. [U-¹⁴C]Acetate (sodium salt) (specific radioactivity, 56mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K.; ethyl methane-sulphonate, DL-glyceric acid and glyoxylic acid monohydrate were from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.; lactate dehydrogenase (crystalline suspension from pig heart, type IV), phosphoenolpyruvate (tricyclohexylamine salt), pyridoxal 5-phosphate and pyruvate kinase (crystalline suspension from rabbit skeletal muscle, type I) were from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Other chemicals were obtained from sources given in the preceding paper (Dunstan et al., 1972).

Bacterial strains

Pseudomonas AM1 (N.C.I.B. 9133) was obtained from the National Collection of Industrial Bacteria, Torrey Research Station, Aberdeen, U.K.

Methods

Maintenance and growth of the organism. The maintenance and growth of cultures of Pseudomonas AM1 have been described (Dunstan et al., 1972). The basal growth medium, without any added carbon source, is referred to as 'salts medium'. Carbon sources included in growth media were used at a concentration of 0.2% except for methanol (0.4%), methylamine (0.4%) and sodium glyoxylate (5mm).

Isolation of mutants and revertants. Mutants and streptomycin-resistant revertants were isolated as described by Dunstan et al. (1972). For the isolation
of revertants of mutants PCT64 and PCT57, streptomycin-resistant organisms were plated on to methanol-agar plates containing 1 mg of streptomycin/ml. For the isolation of revertants of mutant PCT48, streptomycin-resistant mutant cells were plated on to agar plates containing streptomycin (1 mg/ml) and either methanol or ethanol as carbon source.

**Determination of the growth responses of mutant strains.** Initially the growth characteristics of mutant strains were determined by observation of growth in liquid media. When growth occurred the mutant was subcultured at least twice into the same medium (5 ml), by using a wire loop (2 mm diam.). After the second subculture the growth properties of the mutant were confirmed on solid media to ensure that revertants had not arisen during the subcultures.

Sometimes the growth of mutants on liquid media was very poor, and to determine whether or not the carbon source could be used as a growth substrate, alternative methods of testing the growth response were employed.

(a) Replica plating; a master plate with about 100 mutant colonies was replicated on to the solid medium to be tested, and the growth obtained was compared with the growth of wild-type organisms on similar replica plates and with any growth occurring on medium with no added carbon source.

(b) Single colony counts; a suspension of mutant cells in salts medium was spread on to a plate containing the substrate to be tested such that there were about 100 colonies per plate. The growth of single colonies on different media was compared with the growth of single colonies of wild-type cells under the same conditions.

When cultures of mutant cells were used for experiments, the growth responses of these cultures to succinate, methanol, methylvamine and ethanol were tested to ensure that wild-type contaminants were not present.

**Induction of the enzymes involved in the metabolism of C_1 compounds, measurement of oxygen uptake by whole cell suspensions, and bacterial incorporation of [1^4C]acetate.** These methods have been described (Dunstan et al., 1972).

**Enzyme assays.** Sonic extracts were prepared as described by Dunstan et al. (1972). Enzymes were assayed spectrophotometrically in a Unicam SP. 500 spectrophotometer at room temperature; 10 mm light-path silica cells were used. The reference cell contained water.

Hydroxypyruvate reductase (EC 1.1.1.29). This enzyme was assayed by the method described by Dunstan et al. (1972).

Glyoxylate reductase (EC 1.1.1.26). This enzyme was assayed by the same method as hydroxypyruvate reductase, except that 6 μmol of sodium glyoxylate was substituted for lithium hydroxypyruvate.

Serine–glyoxylate aminotransferase. This enzyme was measured by the method of Blackmore & Quayle (1970).

Glycerate kinase (EC 2.7.1.31). This enzyme was assayed by the method of Heptinstall & Quayle (1970).

Methylamine dehydrogenase. This enzyme was assayed by the method of Eady & Large (1968).

Methanol dehydrogenase. This enzyme was assayed by the method of Dunstan et al. (1972).

Glycollate oxidase (EC 1.1.3.1). This enzyme was assayed by the methods described by Kornberg &

---

**Table 1. Growth responses of mutants of Pseudomonas AM1**

Growth responses were determined in liquid media as described in the Experimental section. The relative amounts of growth observed after three serial subcultures in liquid medium are represented by values 0–5 (based on visual estimates).

<table>
<thead>
<tr>
<th>Growth substrate</th>
<th>Strain ...</th>
<th>Wild-type</th>
<th>PCT64</th>
<th>PCT57</th>
<th>PCT48</th>
<th>20BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate</td>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Methanol</td>
<td></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Methylamine</td>
<td></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Formate</td>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oxalate</td>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Methanol + glyoxylate</td>
<td></td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Methanol + glycollate</td>
<td></td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Methanol + acetate</td>
<td></td>
<td>4</td>
<td>—</td>
<td>3</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol + glyoxylate</td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ethanol + glycollate</td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Ethanol + acetate</td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1972
Table 2. Effect of growth substrate(s) on the oxidative capabilities of whole cells of Pseudomonas AM1, and on the activities of the enzymes of the serine pathway in these organisms

Experimental details are given in the Experimental section. Rates of oxidation have been corrected for the O₂ uptake occurring in the absence of substrate.

<table>
<thead>
<tr>
<th>Substrate for oxidation</th>
<th>Growth substrate(s)</th>
<th>Oxygen uptake (μl of O₂/h per mg dry wt.)</th>
<th>Methanol + succinate</th>
<th>Methylamine + succinate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate for oxidation</td>
<td>Growth substrate(s)</td>
<td>Ethanol</td>
<td>Succinate</td>
<td>Methanol</td>
</tr>
<tr>
<td>None</td>
<td>Ethanol Succinate</td>
<td>30</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Methanol</td>
<td></td>
<td>131</td>
<td>27</td>
<td>156</td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
<td>130</td>
<td>26</td>
<td>140</td>
</tr>
<tr>
<td>Methylamine</td>
<td></td>
<td>—</td>
<td>0</td>
<td>127</td>
</tr>
</tbody>
</table>

Specific activity (μmol of substrate used/h per mg of protein)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxypyruvate reductase</td>
<td>28.2</td>
</tr>
<tr>
<td>Serine-glyoxylate amino-transferase</td>
<td>0.2</td>
</tr>
<tr>
<td>Glycerate kinase</td>
<td>0.8</td>
</tr>
<tr>
<td>Methanol dehydrogenase</td>
<td>30.0</td>
</tr>
<tr>
<td>Methylamine dehydrogenase</td>
<td>—</td>
</tr>
</tbody>
</table>

Regulation of enzymes involved in the metabolism of C₁ compounds

Wild-type succinate-grown Pseudomonas AM1 contained low activities of some of the enzymes of the serine pathway (hydroxypyruvate reductase, serine-glyoxylate aminotransferase and glycerate kinase), and methanol and methylamine were oxidized at low rates (Table 2). It was shown that the low oxidation rates for methanol and methylamine were a result of low activities of methanol dehydrogenase and methylamine dehydrogenase. These dehydrogenases were induced when their respective substrates were included in the growth medium together with succinate. By contrast, the enzymes of the serine pathway were not induced by C₁ compounds when succinate was present in the growth medium.

These results suggest that succinate, or a product of succinate metabolism, acts as a catabolite repressor of the serine-pathway enzymes but not of methanol dehydrogenase and methylamine dehydrogenase. For determination of oxidative capabilities, therefore, mutant organisms were grown on mixtures of succinate plus a C₁ compound. For assay of enzymes of the serine pathway, however, mutants were grown to exponential phase on succinate, harvested, and reincubated overnight in medium containing methanol as the sole carbon source.

During these experiments it was observed that the specific activities of the serine-pathway enzymes bore
a well-defined relationship to one another. When the specific activity of hydroxypyruvate reductase was plotted against that for either serine-glyoxylate aminotransferase or glycerate kinase, a straight line was obtained (Fig. 1). No such well-defined relationship was observed between the specific activities of alcohol dehydrogenase and methyamine dehydrogenase nor between the specific activities of these dehydrogenases and of the serine-pathway enzymes. Statistical analysis of the results given in Fig. 1 shows that the three enzymes of the serine pathway (serine-glyoxylate reductase and glycerate kinase) may be regulated in a co-ordinate manner (Jacob & Monod, 1961). However, sophisticated genetic experiments, which are not possible at the present time with this organism, would be required to determine whether or not the genes coding for these enzymes are situated in an operon.

Characterization of mutants of Pseudomonas AM1

Oxidative properties of whole cells of mutants of Pseudomonas AM1. The rates of oxidation of methanol, ethanol, formaldehyde, formate, methyamine and succinate were measured with mutant PCT64, mutant PCT48 and mutant PCT57 grown in media containing succinate plus methanol plus methyamine. In no case were the rates with mutants significantly different from those measured in wild-type organisms.

Mutant PCT64: a mutant with no glycerate kinase. The results given in Table 3 show that this mutant differs from wild-type organisms in its lack of glycerate kinase. This mutant grows well on all substrates tested except for C1 compounds and oxalate, confirming that glycerate kinase is an essential enzyme of the serine pathway as proposed by Large & Quayle (1963). A streptomycin-resistant revertant of this mutant was isolated; this revertant was

![Graph](https://example.com/graph.png)

Fig. 1. Relationship between the specific activities of enzymes of the serine pathway in Pseudomonas AM1

Each point represents specific activities measured in extracts of cells grown on methanol, oxalate, succinate or ethanol or of cells grown on succinate and then induced with methanol for various periods of time. (a) Statistical analysis of the results shows that the best straight line has an intercept of 10.0 on the ordinate ($P < 0.001$). An analysis of variance made by using this regression curve and the regression curve for the best straight line passing through the origin shows that the intercept of 10.0 for the best straight line is not significantly different from zero ($F = 1.604$) (see Cooper, 1969, for statistical methods). (b) The best straight line has an intercept of 4.3 on the ordinate ($P < 0.001$) and an analysis of variance shows that this intercept is not significantly different from zero ($F = 5.524$).
Table 3. Specific activities of enzymes of the serine pathway in extracts of mutants of Pseudomonas AM1

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Methanol plus glycinate</th>
<th>Methanol plus glycinate</th>
<th>Methanol plus glycinate</th>
<th>Methanol plus glycinate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type PCT48</td>
<td>Wild type PCT48</td>
<td>Wild type PCT48</td>
<td>Wild type PCT48</td>
</tr>
<tr>
<td>Succinate</td>
<td>26.0</td>
<td>29.0</td>
<td>13.8</td>
<td>11.7</td>
</tr>
<tr>
<td>Metabolite</td>
<td>2.0</td>
<td>2.3</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Growth substrate</td>
<td>Strain</td>
<td>Strain</td>
<td>Strain</td>
<td>Strain</td>
</tr>
<tr>
<td>Hydroxypyruvate</td>
<td>0.96</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>reductase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerate kinase</td>
<td>0.96</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Glycollate kinase</td>
<td>0.96</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Glycollate kinase</td>
<td>0.96</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Assay methods are described in the Experimental section. Cells were grown either on succinate or on methanol plus glycinate; some succinate-grown cells were then incubated overnight in salts medium in the presence of methanol (0.4%); this procedure was designed to induce the enzymes of the serine pathway.

able to grow on C1 compounds and had regained glycerate kinase activity. The growth of this mutant on ethanol shows that glycerate kinase (and therefore probably the serine pathway) is not involved in the assimilation of ethanol by Pseudomonas AM1. The low activities of serine-glyoxylate aminotransferase, hydroxypyruvate reductase and glycerate kinase in ethanol-grown organisms (Table 2) support this conclusion.

Mutant PCT48. A consideration of growth responses (Table 1) suggests that mutant PCT48 is unable to form glyoxylate when a C1 compound is provided as the potential carbon source. Growth on oxalate can occur because glyoxylate is formed from oxalate by reduction (Blackmore & Quayle, 1970). Ability to grow on oxalate indicates the presence of the serine-pathway enzymes and this was confirmed (for hydroxypyruvate reductase, serine-glyoxylate aminotransferase and glycerate kinase) by using extracts of mutant PCT48 (Table 3). The failure of this mutant to grow on ethanol indicates that an enzyme involved in the pathway for glyoxylate formation from C1 compounds is also essential for the assimilation of ethanol. It has been proposed (Dunstan et al., 1972) that this pathway for ethanol assimilation involves the formation of glyoxylate from acetate (or a derivative) by way of glycylate. The observation that growth of mutant PCT48 on methanol or ethanol occurred when glyoxylate or glycylate, but not acetate, was also included in the growth medium (Table 1) suggests that this mutant is able to form glyoxylate from glycylate but cannot metabolize acetate to glycylate.

Revertants were not obtained from mutant PCT48 even after treatment with N-methyl-\(N'\)-nitro-N-nitrosoguanidine or ethyl methanesulphonate. The possibility that mutant PCT48 is a double mutant cannot therefore be completely excluded. If mutant PCT48 does have two lesions, one lesion may prevent metabolism of acetate to glycylate (thus precluding growth on ethanol), and the second lesion may prevent metabolism of C1 compounds to glyoxylate (thus precluding growth on C1 compounds). If mutant PCT48 is a double mutant of this sort, however, it should have been possible to obtain revertants able to grow with either ethanol or methanol as sole carbon source; such revertants were not obtained. Multiple penicillin screenings, as used in the isolation of mutant PCT48, are likely to lead to selection of stable (non-reverting) mutants such as deletion mutants and mutant PCT48 probably has a single lesion of this type.

Mutant PCT57. Extracts of this mutant contained the enzymes of the serine pathway (Table 3). Mutant PCT57 differs from mutant PCT48 in being able to grow on ethanol. Further, by contrast with mutant PCT48, acetate could replace glyoxylate or glycylate for growth with methanol. This result confirms the
Wild-type cells were grown on ethanol; mutant cells were grown on succinate and were induced overnight in the presence of ethanol (0.2%) and acetate (0.1%). This procedure was designed to allow for the synthesis of inducible enzymes and permeases for C₂ compounds. Washed organisms (6 mg dry wt.) were then preincubated aerobically in 2 ml of salts medium with 1 μmol of acetate at 30°C for 10 min. [U-¹⁴C]Acetate (1.7 μmol; 20 μCi of ¹⁴C) was added at zero time and 1 ml samples were removed at suitable time-intervals into 3 ml of boiling ethanol. The precipitates were extracted with 1 ml ofaq. 20% (v/v) ethanol and the combined extracts were analysed by two-dimensional chromatography. The radioactive areas were located by radioautography and the radioactive compounds were eluted, counted and identified. Full details of procedures are given by Dunstan et al. (1972). Correction was made for quenching and for the background radioactivity of vials (about 45 c.p.m.). The chromatogram background radioactivity was 60–150 c.p.m.; it is given for reference but was not used for correction purposes. A blank sample containing boiled cells and radioactive substrate was treated in an identical manner with the experimental samples; no radioactive compounds were detected in this sample after chromatography and radioautography.

### Radioactivity incorporated into compound (% of total)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Strain ...</th>
<th>Wild-type cells</th>
<th>Mutant PCT48</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time ...</td>
<td>15s</td>
<td>60s</td>
</tr>
<tr>
<td>Glutamate</td>
<td>16s</td>
<td>65.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Compound ‘B’</td>
<td>12.2</td>
<td>8.0</td>
<td>55.0</td>
</tr>
<tr>
<td>Aspartate</td>
<td>8.0</td>
<td>7.1</td>
<td>&lt;4.0*</td>
</tr>
<tr>
<td>Malate</td>
<td>2.8</td>
<td>0.9</td>
<td>&lt;4.0*</td>
</tr>
<tr>
<td>Glycollate</td>
<td>2.8</td>
<td>1.3</td>
<td>&lt;4.0*</td>
</tr>
<tr>
<td>‘Succinate plus fumarate’</td>
<td>3.6</td>
<td>1.0</td>
<td>&lt;4.0*</td>
</tr>
<tr>
<td>Others</td>
<td>38.0</td>
<td>17.0</td>
<td>&lt;4.0*</td>
</tr>
<tr>
<td>Total radioactivity (c.p.m.)</td>
<td>76674</td>
<td>455548</td>
<td>7380</td>
</tr>
</tbody>
</table>

* These compounds were not detected on the radioautogram; the lowest detectable limit under the conditions used was approx. 300 c.p.m. and this value is expressed as a percentage of the total radioactivity incorporated.

suggestion (Dunstan et al., 1972) that enzymes capable of converting acetate into glyoxylate (probably by way of glycylcollate) are present in Pseudomonas AM1.

It is not known whether glyoxylate is formed from a product of the condensation of two C₁ units or by cleavage of a C₄ compound (Large et al., 1962; Harder & Quayle, 1971b). Mutant PCT57 is unable to form glyoxylate from C₁ units, but its properties do not enable a distinction to be made between these two possible origins of glyoxylate during growth of Pseudomonas AM1 on C₁ compounds.

**Mutant 20BL.** The failure of this mutant to grow on ethanol indicates that hydroxypyruvate reductase has a role in the metabolism of ethanol as well as in the pathway for the assimilation of oxalate and of C₁ compounds. The results in Table 1 show that this mutant is able to grow on ethanol, at similar rates to wild-type organisms, when glyoxylate (but not glycylcollate) is included in the growth medium. This growth response indicates that the function of hydroxypyruvate reductase during growth on ethanol (and perhaps a second function during growth on methanol) is the oxidation of glycylcollate to glyoxylate.

Large & Quayle (1963) have shown that the glyoxylate reductase and the hydroxypyruvate reductase of Pseudomonas AM1 are the same enzyme; both activities are present in small amounts in succinate-grown organisms and are induced during growth on C₁ compounds. The specific activity of glyoxylate reductase in extracts of ethanol-grown Pseudomonas AM1 was 2–4 μmol of NADH used/h per mg of protein; this is sufficient to account for the growth rate on ethanol. Although the equilibrium of the reaction catalysed by this enzyme lies towards the formation of glycylcollate, net conversion of glycylcollate into glyoxylate might occur in vivo if the product (glyoxylate) is rapidly removed by metabolism to malate (or to glycine during metabolism of C₁ compounds). No flavoprotein glycollate oxidase could be detected in extracts of Pseudomonas AM1 (see the Experimental section).

**Metabolism of [¹⁴C]acetate by mutant PCT48.** When ethanol-grown wild-type Pseudomonas AM1 is incubated with [¹⁴C]acetate, radioactivity is rapidly incorporated into glutamate, aspartate, malate, glycylcollate, ‘succinate plus fumarate’ (which are not
* This enzyme also catalyses the oxidation of methanol to formaldehyde.

† Mutant PCT57 is unable to catalyse the condensation (Scheme a) or cleavage (Scheme b) reaction to give acetate, glycollate or glyoxylate from C1 compounds. If mutant PCT48 has a single mutation then the product of the reaction is acetate (or a derivative), as shown above. If PCT48 is a double mutant then the product of the condensation or cleavage reaction could be glycollate or glyoxylate instead of acetate.

Scheme 1. *Postulated relationships between pathways for the metabolism of C1 and C2 compounds by Pseudomonas AM1*

(a) Non-cyclic scheme involving direct generation of glycine during growth on C1 compounds; (b) alternative cyclic scheme in which glycine is regenerated by cleavage of a C4 compound. Broken lines represent proposed reactions or series of reactions. Metabolic lesions in the designated mutants are indicated by hatched bars.

Vol. 128
distinguished) and an unidentified compound (compound 'B') (Dunstan et al., 1972). The properties of mutant PCT48, described above, indicate that it is unable to form glycollate from acetate. If this is the case, then radioactive glycollate should not accumulate when mutant cells are incubated with \(^{14}C\)acetate. Further, because the pool of glyoxylate should be negligible in this mutant, radioactive malate should not be formed by the condensation of glyoxylate with acetyl-CoA. The metabolism of radioactive acetate by mutant PCT48 was therefore investigated; the results were compared with those obtained with ethanol-grown wild-type organisms (Table 4). At 15s after addition of [U-\(^{14}C\)]acetate to the mutant cells, radioactivity was detected in only glutamate and compound 'B'. After 60s, radioactivity was also found in 'succinate plus fumarate' but not in glycollate, malate or aspartate. This pattern of incorporation (at 60s) contrasts with that observed in wild-type organisms where similar amounts of radioactivity were incorporated from \(^{14}C\)acetate into glycollate, malate, 'succinate plus fumarate' and aspartate. Although the total amount of radioactivity incorporated into the mutant cells was less than that incorporated into wild-type cells, some label should have been found in glycollate, malate and aspartate if the metabolism of \(^{14}C\)acetate in the mutant is similar to that in wild-type organisms. These results confirm the suggestion that mutant PCT48 is unable to metabolize acetate to glycollate. The radioactive labelling found in compound 'B' indicates that glycollate formation is not required for synthesis of this unidentified compound.

**Discussion**

The results and conclusions presented in this paper are summarized in the two schemes shown in Scheme 1. Both schemes postulate a relationship between the pathways for the metabolism of C1 and C2 compounds in *Pseudomonas AM1*; they differ only in the route by which glycine is formed during growth on C1 compounds. One of the schemes (Scheme 1a) involves non-cyclic formation of glycine from C1 compounds. In the alternative cyclic scheme for glycine regeneration (Scheme 1b; modified from Large et al., 1962) a C4 compound is cleaved to a C2 compound plus acetate (or a derivative of acetate). The metabolic lesions in the mutants of *Pseudomonas* AM1 described in the present paper are the same in the two schemes except for that in mutant PCT57. The growth properties of mutants PCT48, PCT57 and 20BL do not enable a distinction to be made between these two schemes. The properties of mutant PCT64 favour the cyclic scheme (Scheme 1b), because glycerate kinase is essential for conversion of C1 compounds into glycollate by this route. If the alternative, non-cyclic, pathway (Scheme 1a) operates it might be expected that mutant PCT64 would grow slowly on C1 compounds by condensing glyoxylate with acetyl-CoA to give malate. However, it is possible that the non-cyclic scheme does operate in *Pseudomonas* AM1 and that mutant PCT64 is unable to grow on C1 compounds because acetyl-CoA is not readily formed from these growth substrates.

When the results obtained with mutants PCT48 and PCT57 are considered together it can be concluded that acetate (or a derivative) and glycollate are intermediates in the formation of glyoxylate from C1 compounds. If glycollate is an intermediate in this pathway then it is not obvious why radioactive glycollate was absent after incubation of methanol-grown *Pseudomonas* AM1 with \(^{14}C\)methanol (Large et al., 1961). It should be noted, however, that radioactive glycollate was detected at early times when *Hyphomicrobium vagare* was incubated with \(^{14}C\)-methanol (Large et al., 1961). Further, \(^{14}C\)glycollate accumulated when mutant 20BL was incubated with \(^{14}C\)methanol (Heptinstall, 1968). Such accumulation of glycollate would be expected if hydroxypyruvate reductase is required for the oxidation of glycollate to glyoxylate during the assimilation of C1 compounds, as suggested in Scheme 1.

The failure to demonstrate conversion of \(^{14}C\)-acetate into glycollate in mutant PCT48 confirms the proposal that metabolism of acetate to glycollate is essential for ethanol assimilation in *Pseudomonas* AM1 (Dunstan et al., 1972). The proposed pathway for ethanol assimilation is further supported by the growth responses of mutant PCT57, which demonstrate that enzymes capable of catalysing the conversion of acetate into glyoxylate are present in this organism. Preliminary attempts to demonstrate conversion of acetate into glycollate in extracts of *Pseudomonas* AM1 have not been successful.

During growth on succinate, serine is synthesized by a pathway involving phosphoserine phosphatase (Heptinstall & Quayle, 1970). A mutant (20S) lacking this enzyme does not grow on succinate, lactate or ethanol (Harder & Quayle, 1971a). Consideration of Scheme 1 might suggest that serine could be synthesized by way of glyoxylate and glycine during growth on ethanol. In this case C1 units for serine synthesis could be provided by the oxidative decarboxylation of glyoxylate (this reaction has been demonstrated in *Pseudomonas* AM1 by Harder & Quayle, 1971b). However, serine-glyoxylate aminotransferase is not induced during growth on ethanol (Table 2) and thus the phosphorylated pathway is the only possible route for serine synthesis.

It should be noted that most of the reactions in the proposed pathway for the assimilation of ethanol are also involved in the proposed pathway for methanol assimilation. The abundance of pink methanol-utilizing bacteria in Nature is probably related to
their relatively high growth rate on C₁ compounds (an unusual characteristic in bacteria). These bacteria grow slowly on ethanol compared with many other soil organisms. It is therefore suggested that growth of these bacteria on ethanol is not important in Nature and that when growth on this compound does occur this is a consequence of the reactions common to C₁ and C₂ metabolism as postulated in Scheme 1.

We thank the Science Research Council for a research grant to C. A. and for a research studentship for P. M. D.

References
Harder, W. & Quayle, J. R. (1971a) Biochem. J. 121, 753–762
Large, P. J. & Quayle, J. R. (1963) Biochem. J. 87, 386–396
Large, P. J., Peel, D. & Quayle, J. R. (1962) Biochem. J. 82, 483–488