The Microbial Metabolism of Acetone

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Four Gram-positive bacteria have been isolated from separate soil samples by enrichment culture with acetone as sole source of carbon. Whole cells of all strains grown on acetone rapidly oxidized acetone, acetol and methylglyoxal, and three of the four also oxidized isopropanol. The patterns of induced enzymes in cell extracts are compatible with the oxidation sequence: isopropanol → acetone → acetol → methylglyoxal → pyruvate. Although an enzyme system capable of converting acetone into acetol has not been detected, the inclusion of acetol in the pathway is supported by the results of studies with whole cells and [14C]acetone. The proposed pathway of acetone metabolism is contrasted with evidence for an alternative, but not fully understood, pathway used by Mycobacterium vaccae JOB5.

INTRODUCTION

Although several groups have studied acetone degradation by animals and microorganisms, the precise metabolic routes involved and the nature of the carbon fragments that enter the central metabolic pathways are still unclear. Sakami & Lafaye (1951) and Rudney (1954) reported studies with the rat which indicated a cleavage of acetone metabolites to yield acetate and formate while pyruvate and lactate were formed directly. Studies with microorganisms have also indicated the probable cleavage of the acetone carbon skeleton into organic C1 and C2 fragments. Early work (Supniewski, 1923; Goepfert, 1941) indicated the formation of formate and formaldehyde by species of Bacillus and Fusarium. The oxidation of acetone by a soil diphtheroid (Levine & Krampitz, 1952) yielded acetaldehyde and a C1 fragment. Vestal & Perry (1969) showed that acetone was an intermediate in propane metabolism by Mycobacterium vaccae JOB5 and that further metabolism yielded acetate and CO2, resulting in the induction of isocitrate lyase (EC 4.1.3.1) in propane-grown cells. Lukins & Foster (1963) have, like others, provided evidence that acetol (1-hydroxyacetone) is an intermediate in acetone degradation. However, in their studies with Mycobacterium smegmatis, they presented no clear evidence to indicate how acetol is further metabolized.

Previous workers have relied heavily on whole-cell oxidation studies and radiochemical techniques. In this study, extracts have also been used to investigate the enzymology of a number of isolates grown with acetone and related compounds as sole sources of carbon. A route for the conversion of acetone into a central metabolic pathway intermediate is proposed.

METHODS

Bacterial strains. Strains A1 and A2 were obtained from Aberystwyth soil and estuarine mud (Penarth), respectively, by elective culture with acetone (0.2%, v/v) as sole source of carbon. Strains S1 and S2 were obtained by elective culture with acetone and isopropanol, respectively, from soil samples taken in the

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Sittingbourne area. All four organisms display morphological and biochemical characteristics which would appear to place them in the *Corinobacterium* group. All are Gram-positive; young cultures consist of irregular rods with complex angular arrangements which give rise to cocci in older cultures. All are non-motile, catalase-positive, oxidase-negative and hydrolyse starch but not gelatin or arginine. Strains A2, SA1 and SPI show weak surface growth in glucose media without acid production, while strain A1 produces acid from glucose oxidatively. All grow on Koser's citrate medium and all except strain SPI produce nitrite from nitrate. Good growth was obtained on King's media glucose oxidatively. The strains were maintained at 4 °C on nutrient agar slants grown at 30 °C for 72 h. *Mycobacterium vaccae* JOB5 was a generous gift from Dr J. J. Perry.

**Growth conditions.** Cultures were grown aerobiocically at 30 °C on a rotary shaker (150 rev. min⁻¹). Basal media contained (g 1⁻¹): KH₂PO₄, 2; Na₂HPO₄, 4; (NH₄)₂SO₄, 1; and 4 ml trace metal stock solution (Rosenberger & Elsdon, 1960). Carbon sources were added at 2 g 1⁻¹ either before sterilization of the medium or, in the case of volatile compounds, aseptically after sterilization. Cultures were usually grown according to the following procedure: 25 ml medium in a 100 ml conical flask was inoculated from a slope and after 48 h growth this was used to inoculate 100 ml medium in a 250 ml conical flask. After growth to the late-exponential phase this culture was transferred to 11 medium in a 2 l conical flask which was similarly incubated and the organisms were harvested in the late-exponential phase of growth by centrifugation (16000 g, 3 °C, 20 min). Cell pellets were washed by resuspension in 50 mM-phosphate buffer, pH 7-1, followed by centrifugation.

*Preparation of cell extracts.* Washed cells resuspended in 50 mM-phosphate buffer, pH 7-1 (2 x cell vol.) were disrupted by one passage through a French press with a pressure difference at the orifice of 138 MPa. Broken cell suspensions were centrifuged (36000 g, 3 °C, 30 min). The protein content of extracts was determined by the method of Gornall et al. (1949).

**Buffers.** The sodium/potassium phosphate buffer routinely used was prepared by dissolving 4 g Na₂HPO₄ and 2 g KH₂PO₄ in 1 l distilled water, giving a pH of 7-1. Other buffers were obtained by adjustment of a solution of the appropriate compound to the required pH with 1 M-NaOH or 1 M-HCl and diluting to the required concentration.

**Oxygen-uptake measurements.** O₂ consumption by cell suspensions or by cell extracts was determined by conventional Warburg manometry or with a Clark-type O₂ electrode in an agitated vessel maintained at 30 °C (Yellow Springs Instrument Co.).

**Estimation of compounds.** Methylglyoxal was assayed by the method of Gawehn & Bergmeyer (1974). Pyruvate was assayed either by the method of Friedemann & Haugen (1943) or by measuring the total change in absorbance at 340 nm effected by L-lactate dehydrogenase in the presence of excess NADH (Coz & Lamprecht, 1974).

**Enzyme activities.** Propan-1,2-diol dehydrogenase (EC 1.1.1.41) was assayed by measuring the increase in absorbance at 340 nm when the test extract was added to a 1 cm-lightpath cuvette that contained, in 1 ml: glycine/NaOH buffer, pH 10 (90 μmol), NAD (0·5 μmol) and propan-1,2-diol (2 μmol).

Isopropanol dehydrogenase (EC 1.1.1.5) was assayed by measuring the decrease in absorbance at 340 nm when the test extract was added to a 1 cm-lightpath cuvette that contained, in 1 ml: KH₂PO₄/Na₂HPO₄ buffer, pH 7-0 (90 μmol), NADH (0·15 μmol) and acetone (1 μmol). Observed reaction rates were corrected for endogenous oxidation of NADH.

Acetol (1-hydroxyacetone) dehydrogenase (EC 1.1.1.41) was assayed by measuring the increase in absorbance at 340 nm when the test extract was added to a 1 cm-lightpath cuvette that contained, in 1 ml: glycine/NaOH buffer, pH 10 (90 μmol), NAD (0·5 μmol) and acetol (2 μmol).

Methylglyoxal (pyruvaldehyde) dehydrogenase (EC 1.1.1.41) was assayed in the same manner as acetal dehydrogenase but with methylglyoxal as substrate. Endogenous oxidation of NADH was negligible under these assay conditions.

**Isocitrate lyase (EC 4.1.3.1).** was assayed according to the procedure of Reeves et al. (1971).

Glyoxalase I (EC 4.4.1.5) was assayed according to the following procedure. A reaction mixture, incubated at 30 °C, contained, in 5 ml: KH₂PO₄/Na₂HPO₄ buffer, pH 7-1 (300 μmol), reduced glutathione (15 μmol), cell extract (5 to 10 mg protein) and methylglyoxal (5 μmol). After incubation for 30 min, reactions were stopped by the addition of 1 ml 2 M-HCl and, after one cycle of freezing and thawing, protein was removed by centrifugation (36000 g, 3 °C, 15 min). The supernatant was readjusted to pH 7 (1 ml 2 M-NaOH) and residual methylglyoxal was then measured by the procedure of Gawehn & Bergmeyer (1974).

Methyl acetate hydrolyse (EC 3.1.1.1) was assayed by measuring the rate of ester hydrolysis in reaction mixtures containing, in 8 ml: KH₂PO₄/Na₂HPO₄ buffer, pH 7-1 (700 μmol), cell extract (2 to 20 mg protein) and methyl acetate (40 μmol). At intervals, 1 ml samples of the reaction mixture were taken and the residual substrate was measured by the method of Cain (1961).

One enzyme unit is defined as the consumption of 1 μmol substrate or the formation of 1 μmol product min⁻¹.
Preparation of samples for chromatography. Reactions were usually terminated by acidification with 2.5 M HCl and protein was then removed by centrifugation. Reaction products were extracted using an appropriate organic solvent.

For the preparation of dinitrophenylhydrazone derivatives, deproteinized reaction mixtures were incubated with 2,4-dinitrophenylhydrazine reagent (Friedemann & Haugen, 1943) and the derivatives were extracted into ethyl acetate. Acidic 2,4-dinitrophenylhydrazones were differentially extracted into 10% (w/v) Na2CO3 solution, followed by acidification and re-extraction into ethyl acetate. Ethyl acetate extracts were dried over anhydrous Na2SO4 and evaporated to a small volume.

Acetol in reaction mixtures was converted into 3-hydroxy-2-methylquinoline by reaction with excess 2-aminobenzaldehyde at pH 12 and 60 °C for 30 min (Baudisch, 1918). After cooling, the solution was acidified with HCl, filtered and again made alkaline with NaHCO3. The derivative was extracted into diethyl ether, dried and evaporated to a small volume.

Chromatography. All thin-layer chromatography (t.l.c.) was carried out on glass plates coated with Kieselgel G (Merck) to a thickness of 0.25 mm and activated for 30 min at 110 °C. For 2,4-dinitrophenylhydrazones of neutral compounds, chromatograms were developed with either solvent 1 [hexane/ethyl formate (4 : 1, v/v)] or solvent 2 [toluene/dioxan/acetic acid (90 : 25 : 4, by vol.)]. For 2,4-dinitrophenylhydrazones of acidic compounds, chromatograms were developed with solvent 3 [petroleum ether (b.p. 60 to 80 °C)/ethyl formate/propanionic acid (70 : 30 : 3, by vol.)]. T.l.c. of 3-hydroxy-2-methylquinoline was carried out with solvent 2, solvent 4 [chloroform/methanol/acetic acid (32 : 2 : 1, by vol.)], solvent 5 [butanol-1-ol/pyridine/water (6 : 4 : 3, by vol.)] or solvent 6 [ethanol/ammonia/water (8 : 1 : 1, by vol.)]. Paper chromatograms of 2,4-dinitrophenylhydrazones of acidic compounds were developed with solvent 7 [butanol-1-ol/ethanol/ammonium carbonate buffer (40 : 11 : 9, by vol.)] (Dagley et al., 1952).

Radioactivity measurements. Radioactive areas of chromatograms were located either by autoradiography or with a thin-layer radiocromatogram scanner (Panax Nucleonics). Liquid scintillation counting of 2,4-dinitrophenylhydrazones spots scraped from the t.l.c. plates was performed in an SL30 liquid scintillation spectrometer (Intertechnique) using NE260 liquid scintillation fluid (Nuclear Enterprises) which solubilized 2,4-dinitrophenylhydrazones. Radioactive bacteria were collected on membrane filters (0.22 μm pore size, Millipore), thoroughly washed with 50 mM-phosphate buffer, pH 7.1, and the air-dried filters were counted in an automatic planchet counter (Nuclear Enterprises). All radioactivities were expressed as c.p.m. and were corrected for background radiation but not for quenching or counter efficiency.

Partial purification of methylglyoxal dehydrogenase. Crude extract (380 mg protein) was chromatographed at 3 °C on DEAE-cellulose (2.5 x 9 cm column) and eluted with a gradient of 0 to 0.7 M KCl in 50 mM-phosphate buffer, pH 7.1, containing 10% (v/v) ethanol. Active fractions were pooled and used directly for experimental study.

Chemicals. Acetol (1-hydroxyacetone) and 2-aminobenzaldehyde were obtained from Aldrich Chemical Co.; acetol was redistilled before use. Methylglyoxal was obtained from Sigma as a 40% (v/v) solution and was purified by chromatography on a column of Dowex 1 chloride (1-X8, 200 to 400 mesh). Fractions containing methylglyoxal were detected with 2,4-dinitrophenylhydrazine reagent and the pooled solution was assayed according to the procedure of Gawehn & Bergmeyer (1974). 3-Hydroxy-2-methylquinoline was prepared by the method of Baudisch (1918) and glyoxalase I (EC 4.4.1.5), L-lactate dehydrogenase (EC 1.1.1.27), NAD+, NADP+, NADH and NADPH were from Boehringer.

RESULTS

The isolation of bacterial strains capable of growth on acetone is described in Methods. Strain A1 has been the principal experimental subject but corroborative evidence obtained with the other strains is reported in appropriate sections.

A study of the spectrum of substrates that could be utilized by our isolates as sole sources of carbon is of little assistance as an indicator of the route of acetone degradation since some of the compounds, though structurally similar to acetone, are unlikely to be metabolically related. For example, strain A1 grew on a wide range of C3 compounds, including propan-1,2-diol, isopropanol, acetol, methyl acetate, pyruvate and lactate. Methylglyoxal, however, was not used as a growth substrate, even at a concentration of 0.02% (w/v), which was tested simultaneously with 0.05 and 0.1% (w/v) in view of the known inhibitory effect of methylglyoxal on bacterial growth (Együd & Szent-Györgyi, 1966). In addition, methanol, acetate and methyl ethyl ketone (2-butanol) could serve as carbon sources.
Fig. 1. Oxidation of various compounds by strain A1 grown on acetone. Warburg flasks contained 5 mg dry wt bacteria in 1-65 ml 50 mM-phosphate buffer, pH 7-0, 0·25 ml 20 mM substrate tipped from the side-arm and 0·1 ml 20% (w/v) KOH in the centre well. Oxygen uptake was measured at 30 °C. The uptake in a flask lacking substrate (19 μl O₂ h⁻¹) has been subtracted. Substrates were: •, acetone; ○, acetol; □, isopropanol; ■, methylglyoxal; △, methyl acetate; ▲, methanol and acetate; ▼, pyruvate. There was no significant oxidation of dl-lactate or succinate by acetone-grown cells.

Oxidation of compounds by whole cells

Whole cells of strains A1, A2, SA1 and SP1, grown with acetone as sole source of carbon, rapidly oxidized the growth substrate and the related compounds isopropanol, acetol and methylglyoxal. Methyl acetate, methanol, acetate and dl-lactate were, by comparison, oxidized slowly. Results for strain A1 are shown in Fig. 1. Cells grown on succinate did not rapidly respire acetone or the three related compounds, implying that the ability to metabolize acetone is inducible.

Enzyme activities in cell extracts

The whole-cell oxidation studies allowed a sequence of putative metabolites to be arranged in order of increasing oxidation state: isopropanol → acetone → acetol → methylglyoxal. Because of the structural simplicity of the compounds, enzyme-catalysed steps converting isopropanol into methylglyoxal can easily be postulated and include an initial dehydrogenation, attack on acetone by a mono-oxygenase and two further dehydrogenations.

Alternative routes may be postulated for the further metabolism of methylglyoxal and include conversion into lactate by the action of glyoxalase I and glyoxalase II (Racker, 1951) or direct oxidation to pyruvate (Monder, 1967).

A comparison of enzyme activities in extracts of strain A1 grown on acetone and succinate or acetate shows clearly the inducible nature of dehydrogenases that are active towards isopropanol, acetol and methylglyoxal (Table 1). The indication here that methylglyoxal is further metabolized directly to pyruvate is corroborated by the absence of glyoxalase I from extracts of acetone-grown cells.

Our inability to demonstrate the presence of an acetone mono-oxygenase in extracts of strain A1 has been repeated in our other strains (Table 2) and may indicate a highly unstable enzyme. It does, however, make the direct identification of the product of any such oxygenation in vitro impossible. Two possible alternative oxygen-introducing reactions can be en-
Table 1. Enzyme activities in extracts of strain A1 grown on various compounds

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cells grown on:</th>
<th>Specific activity [units (mg protein)^{-1}]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methyl acetate</td>
<td>Acetone</td>
</tr>
<tr>
<td>Propan-1,2-diol dehydrogenase</td>
<td>0.02</td>
<td>NA</td>
</tr>
<tr>
<td>Isopropanol dehydrogenase</td>
<td>0.21</td>
<td>0.02</td>
</tr>
<tr>
<td>Acetone mono-oxygenase</td>
<td>__</td>
<td>NA</td>
</tr>
<tr>
<td>Acetol dehydrogenase*</td>
<td>0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>Methylglyoxal dehydrogenase</td>
<td>0.40</td>
<td>0.42</td>
</tr>
<tr>
<td>Glyoxalase I</td>
<td>&lt;0.004</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>Isocitrate lyase</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Methyl acetate hydrolase</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

NA, Not assayed; --, not detectable.
* Maximum values; they may include an element of methylglyoxal dehydrogenase.

visaged. The first, catalysed by a methylhydroxylase, would yield acetol and is indirectly supported by the observed enzyme activities. The second, addition of oxygen to the ketone with a rearrangement to form methyl acetate, would be a biological manifestation of the Baeyer-Villiger reaction. Such reactions are quite widely distributed in Nature and are involved in the oxygenation of tridecan-2-one to undecyl acetate (Britton & Markovetz, 1977) and acetophenone to phenyl acetate (Cripps, 1975), the elimination of the side-chain of progesterone (Rahim & Sih, 1966) and the formation of lactones from a variety of cyclic ketones (Trudgill, 1978). This second route, which would certainly yield the C₂ and C₁ fragments indicated as the products of cleavage of the acetone carbon skeleton by a number of workers, is inoperative in our strains as demonstrated by the basal activity of methyl acetate hydrolase and isocitrate lyase in extracts of cells grown on acetone or acetol. These are in marked contrast to the activity of isocitrate lyase in cells grown on methyl acetate or acetate and of methyl acetate hydrolase in cells grown on methyl acetate (Table 1, Table 2).

Indirect evidence for the formation of acetol from acetone

All our attempts to demonstrate by conventional means the presence of an acetone mono-oxygenase failed. These included tests with extracts of all our organisms in which either substrate-stimulated NADH or NADPH oxidation was measured in conjunction with substrate-stimulated O₂ consumption. Preparation of extracts under a variety of conditions and the inclusion of putative stabilizing or activation agents all failed to give any detectable activity.

Two alternative approaches were therefore employed to demonstrate the direct conversion of acetone into acetol. Firstly, whole cells of strain A1 were incubated with 10 mM concentrations of acetone, acetol or methylglyoxal in the presence of 5 mM-sodium arsenite. In all three cases significant accumulation of pyruvate occurred, the rate of accumulation being more rapid the nearer the compound to pyruvate in metabolic terms (Fig. 2). While this provides supporting evidence for a sequence, acetone → acetol → methylglyoxal → pyruvate, it does not constitute proof of a direct hydroxylation of acetone to acetol.

In a second experiment, the fate of [2-¹⁴C]acetone was observed in whole cells over a relatively short period in the absence of any inhibitor. Cells were removed from samples of the reaction system by filtration and 2,4-dinitrophenylhydrazones were prepared from susceptible metabolites and separated into neutral and acidic fractions. Thin-layer chromatography followed by autoradiography, elution and counting allowed radioactive products to be identified and estimated. The accumulation of [¹⁴C]pyruvate was accompanied by a transient appearance of [¹⁴C]acetol, thus providing confirmatory evidence for the conversion
Table 2. *Enzyme activities in extracts of strains A2, SA1 and SP1 grown on various compounds*

Enzymes were assayed as described in Methods.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Strain A2</th>
<th>Strain SA1</th>
<th>Strain SP1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cells grown on:</td>
<td></td>
<td>Cells grown on:</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>acetate</td>
<td>Acetate</td>
</tr>
<tr>
<td>Isopropanol dehydrogenase</td>
<td>0.12</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Acetone mono-oxygenase</td>
<td>—</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Acetol dehydrogenase</td>
<td>0.06</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Methylglyoxal dehydrogenase</td>
<td>0.35</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Glyoxalase I</td>
<td>&lt;0.004</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Isocitrate lyase</td>
<td>0.03</td>
<td>NA</td>
<td>0.11</td>
</tr>
<tr>
<td>Methyl acetate hydrolase</td>
<td>0.28</td>
<td>9.4</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, Not assayed; —, not detectable.
Fig. 2. Accumulation of pyruvate from acetone, acetol and methylglyoxal by whole cells of strain A1 in the presence of 5 mM-sodium arsenite. Reaction vessels (250 ml conical flasks), incubated in a gyrating shaker at 30 °C, contained, in 100 ml 50 mM-phosphate buffer, pH 7·0, bacteria (36 mg dry wt), 5 mM-sodium arsenite and 10 mM substrate as indicated. Samples (50 μl) were withdrawn from the flasks at intervals and added to 1 cm-lightpath cuvettes containing 90 μmol phosphate buffer, pH 7·0, 0·15 μmol NADH and 0·3 units L-lactate dehydrogenase. The amount of pyruvate accumulated from acetone (△), acetol (○) and methylglyoxal (○) was calculated from the stimulated decrease in absorbance at 340 nm. Values have been corrected for an endogenous control flask from which substrate was omitted.

Fig. 3. Distribution of radioactivity from [2-14C]acetone in metabolites of strain A1 grown on acetone. Reaction vessels (50 ml conical flasks), incubated in a metabolic shaker at 30 °C, contained, in 8 ml 50 mM-phosphate buffer, pH 7·0, bacteria (3·5 mg dry wt), 6 μmol [2-14C]acetone (sp.act. 0·83 mCi mmol⁻¹, 30·7 MBq mmol⁻¹) and 10 μmol unlabelled acetol. Samples (1 ml) were removed at intervals and the bacteria were harvested on 0·22 μm pore-size membrane filters. The filters were washed by the passage of 2 ml 50 mM-phosphate buffer, pH 7·0. The washings and filtrate were combined and incubated at 30 °C for 30 min with 0·5 ml 2,4-dinitrophenylhydrazine reagent (0·1 %, w/v, in 2 M-HCl). Derivatives were extracted with 0·5 ml ethyl acetate and acidic derivatives were differentially extracted from the ethyl acetate solution with 1 ml 10 % (w/v) Na₂CO₃. Acidification of the Na₂CO₃ solution allowed the acidic derivatives to be re-extracted into ethyl acetate. Ethyl acetate solutions were dried over anhydrous Na₂SO₄ and 20 μl samples were chromatographed and counted as described in Methods. Radioactivity is expressed as the percentage distribution of counts in acetone (○), acetol (●), pyruvate (△) and bacteria (●).

of acetone into acetol (Fig. 3). No significant radioactivity co-chromatographed with the 2,4-dinitrophenylhydrazone of methylglyoxal.

Further support for the direct involvement of acetol has come from experiments with strains SA1 and SP1 in which bacteria (40 mg dry wt) were incubated with 200 μmol [1,3-14C]acetone (sp.act. 0·75 μCi mmol⁻¹, 28 kBq mmol⁻¹) and 300 μmol acetol in 10 ml 50 mM-phosphate buffer, pH 7·0, on a gyrating shaker at 30 °C for 2 h. Bacteria were removed from the reaction systems by centrifugation (10000 g, 4 °C, 10 min) and acetol in the supernatant was converted into 3-hydroxy-2-methylquinoline as described in Methods (Baudisch, 1918). Chromatographic analysis of the product with solvent systems 2, 4, 5 and 6 yielded a radioactive spot that co-chromatographed with authentic 3-hydroxy-2-methylquinoline in all cases.

**Conversion of acetol into pyruvate by extracts of strain A1**

Preliminary experiments established that two dehydrogenation steps, acetol → methylglyoxal and methylglyoxal → pyruvate, are catalysed by discrete enzymes of strain A1.
Table 3. Pyruvate formation from acetol by dialysed extract of strain A1 grown on acetone

<table>
<thead>
<tr>
<th>Acetol added (µmol)</th>
<th>Pyruvate produced (µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>3</td>
<td>2.74</td>
</tr>
<tr>
<td>6</td>
<td>5.44</td>
</tr>
</tbody>
</table>

---, Not detectable.

Table 4. Stoichiometry of NAD+ reduction and pyruvate formation from methylglyoxal by partially purified methylglyoxal dehydrogenase

<table>
<thead>
<tr>
<th>Methylglyoxal added (µmol)</th>
<th>Pyruvate produced (µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>0.25</td>
<td>0.26</td>
</tr>
<tr>
<td>0.50</td>
<td>0.46</td>
</tr>
<tr>
<td>0.75</td>
<td>0.75</td>
</tr>
</tbody>
</table>

---, Not detectable.

Methylglyoxal dehydrogenase was stable in crude extracts stored at 3 °C while acetol dehydrogenase was completely inactivated within 24 h. Inclusion of 10% (v/v) ethanol in crude extracts effectively stabilized the acetol dehydrogenase with no significant loss in activity at 3 °C over a period of 70 h, although its inclusion in enzyme purification procedures has not, as yet, resulted in the recovery of significant activity from DEAE-cellulose chromatography.

Incubation of crude extract, dialysed for 18 h in the presence of 10% (v/v) ethanol, with acetol in the presence of an excess of NAD+ at alkali pH resulted in substantial conversion of the acetol into pyruvate (Table 3). At lower pH values the conversion was less complete.

Stoichiometry of methylglyoxal oxidation by partially purified methylglyoxal dehydrogenase

Methylglyoxal dehydrogenase, purified sixfold by DEAE-cellulose chromatography and devoid of acetol dehydrogenase activity, was used to establish the stoichiometry of this dehydrogenase reaction. The addition of each µmol methylglyoxal was accompanied by the reduction of 1.04 µmol NAD+ and the formation of 0.95 to 1.08 µmol pyruvate (Table 4).
Fig. 4. Pathway of metabolism of isopropanol and acetone by strains A1, A2, SA1 and SP1.

glyoxalase I from cell extracts is indicative of a single fate for methylglyoxal, i.e. direct oxidation to pyruvate.

*Absent from strain SA1.

- denotes metabolic reactions that do not occur in the strains tested.

Enzyme activities in extracts of strains A2, SA1 and SP1

Dehydrogenases active with isopropanol, acetol and methylglyoxal as substrates were all induced in strains A2 and SP1 grown with acetone as sole source of carbon (Table 2). Key enzymes that might be indicative of alternative metabolic routes – glyoxalase I, isocitrate lyase, methyl acetate hydrolase – were present at activities comparable with those in extracts of cells grown on the control substrate succinate or were present at levels too low to be significant. Activities of isocitrate lyase and methyl acetate hydrolase in extracts of cells grown on acetate and methyl acetate reinforce the conclusion that strains A2, SA1 and SP1 use the same metabolic sequence for the conversion of acetone into pyruvate as has been established in our more detailed study of strain A1 (Fig. 4). The inclusion of isopropanol in the metabolic scheme is justified by the presence of the same induced enzyme pattern irrespective of whether strain A1 was grown on isopropanol or acetone. Strain SA1 differed only in not possessing isopropanol dehydrogenase activity when grown on acetone.

Enzyme activities in extracts of Mycobacterium vaccae JOB5

Extracts of Mycobacterium vaccae JOB5 grown on acetone contained an induced isopropanol dehydrogenase as did those from strains A1, A2 and SP1. However, in contrast to our isolates, growth of JOB5 with acetone failed to induce dehydrogenases active towards acetol and methylglyoxal but did induce an isocitrate lyase activity comparable with that found in extracts of acetate-grown cells (Table 5).

Although Vestal & Perry (1969) have produced evidence to show that Mycobacterium vaccae JOB5 converts acetone into acetol, the further metabolism of acetol in this organism appears to follow a route that differs from the pathway established for strains A1, A2, SA1 and SP1.

Discussion

Previous studies of the metabolism of acetone, isopropanol and propane have indicated that in some instances the C₃ skeleton is cleaved to yield organic C₁ and C₂ fragments before
Table 5. Enzyme activities in extracts of *Mycobacterium vaccae* JOB5 grown on various compounds

Enzymes were assayed as described in Methods.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity [units (mg protein)^{-1}]</th>
<th>Cells grown on:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
</tr>
<tr>
<td>Isopropanol dehydrogenase</td>
<td>0.29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Acetone mono-oxygenase</td>
<td>---</td>
<td>NA</td>
</tr>
<tr>
<td>Acetol dehydrogenase</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Methylglyoxal dehydrogenase</td>
<td>0.034</td>
<td>0.025</td>
</tr>
<tr>
<td>Glyoxalase I</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Isocitrate lyase</td>
<td>0.33</td>
<td>0.24</td>
</tr>
<tr>
<td>Methyl acetate hydrolase</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

NA, Not assayed; ---, not detectable.

the entry of carbon into the central metabolic pathways (Supniewski, 1923; Goepfert, 1941; Sakami, 1950; Levine & Krampitz, 1952; Rudney, 1954; Vestal & Perry, 1969). However, while acetol (1-hydroxyacetone) has been implicated as an intermediate in the degradation of acetone, the mechanism of its formation and the nature of the subsequent metabolic steps remain unclear.

Isolates A1, A2, SA1 and SP1 although obtained from different locations display taxonomic similarities and common induced enzyme patterns (Table 1, Table 2) which are indicative of an ability to convert acetol directly into pyruvate, with methylglyoxal as an intermediate. Alternative metabolic sequences, such as the conversion of methylglyoxal directly into lactate as a precursor of pyruvate and cleavage to organic $C_1$ and $C_2$ fragments, are not supported by the results.

Because of the close similarity in enzyme induction patterns for all four organisms one only, strain A1, was chosen for detailed study. The key dehydrogenation steps from acetol to methylglyoxal and methylglyoxal to pyruvate are catalysed by discrete enzymes. Methylglyoxal dehydrogenase has previously been described in sheep liver (Monder, 1967) and is involved in one of the alternative routes for the further degradation of methylglyoxal derived from aminoacetone by a strain of *Pseudomonas* (Higgins & Turner, 1969). As with the enzyme from both sources, methylglyoxal dehydrogenase from strain A1 has an alkali pH optimum, is dependent on a pyridine nucleotide electron acceptor and catalyses a quantitative conversion of methylglyoxal into pyruvate (Table 4).

The initial step in acetone utilization has not been demonstrated in extracts of any of our strains. Several possibilities exist, primarily supported by in vivo studies with $^{14}$C acetone. Rudney (1954), for example, has proposed the hydration of the enol form of acetone to yield propane-1,2-diol which would then, by dehydrogenation, form acetol. While we have not investigated the possibility of propan-1,2-diol acting as an intermediate between acetone and acetol, the very low dehydrogenase activity towards this compound in extracts of acetone-grown strain A1 (Table 1) makes this unlikely, a view supported by Levine & Krampitz (1952) in their studies with an acetone-grown soil diphtheroid.

The alternative, an oxygen-inserting attack on acetone, could be of two types. In the first place a Baeyer-Villiger type of oxygenation would yield methyl acetate; this, however, may be discounted in view of the failure of growth with acetone to induce a methyl acetate hydrolase (Table 1, Table 2). Hydroxylation of acetone, to form acetol, is a more attractive postulate although we have been unable to demonstrate any such activity in cell extracts. This may be explained by extreme lability of the enzyme concerned or a requirement for a structural organization that is lost upon cell rupture.
In the absence of a demonstrable enzyme activity, proof of the conversion of acetone into acetol was sought from studies with whole cells of strain A1. Incubation of washed acetone-grown cells with [2-14C]acetone in the presence of carrier acetol resulted in the transient accumulation of radioactivity in the acetol (Fig. 3) as well as accumulation in pyruvate and cell material. The shape of the radioactive acetol distribution curve is that expected if the compound is a true metabolic intermediate rather than a side-metabolite. This direct involvement of acetol is also supported by an alternative approach used with strains SA1 and SP1 in which radioactive acetol was trapped as 3-hydroxy-2-methyl-quinoline. Taken together, the accumulated results allow the metabolic pathway shown in Fig. 4 to be postulated in which all reaction steps, with the exception of the conversion of acetone into acetol, have been characterized.

Although it is not our purpose to describe here a study of acetone metabolism by Mycobacterium vaccae JOB5, some limited observations support the findings of Vestal & Perry (1969) and enable us to contrast this organism with our isolates. Extracts of Mycobacterium vaccae JOB5 grown with acetone as sole source of carbon contain a high induced activity of isocitrate lyase. While the assumption that this is indicative of the degradation of acetone to acetate without the intermediate participation of pyruvate is a superficial interpretation of the regulation of isocitrate lyase (Kornberg, 1966; Herman & Bell, 1970; Flavell & Woodward, 1971; Sariaslani et al., 1975; Griffiths, 1979), taken in conjunction with additional observations of Vestal & Perry (1969) who could find no evidence for the significant conversion of [2-14C]isopropanol into pyruvate and the activities of relevant enzymes in extracts of acetone-grown cells (Table 5), it is clear evidence that an alternative pathway of acetone and isopropanol metabolism exists. One postulated pathway that would give rise to organic C4 and C6 fragments would involve a Baeyer-Villiger type of oxygenation of acetone to form methyl acetate which, by the action of an appropriate esterase, would give rise to methanol and acetate. Our failure to detect these enzymes in extracts of Mycobacterium vaccae JOB5 is indicative that such a route is probably not operative and the pathway of metabolism in this organism remains distinctive and unknown.

REFERENCES

control in *Acinetobacter* sp. I. Effect of C\textsubscript{4} versus C\textsubscript{2} and C\textsubscript{3} substrates on isocitrate lyase synthesis. *Canadian Journal of Microbiology* 16, 769–774.


