EVIDENCE FOR ALTERNATE PATHWAYS FOR THE OXIDATION OF GLUCOSE BY PSEUDOMONAS AERUGINOSA[1]

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Species of *Pseudomonas* accumulate gluconic and 2-ketogluconic acids when grown in a medium containing glucose (Norris and Campbell, 1949; Stubbs *et al.*, 1940; Entner and Stanier, 1951). In addition, cell-free preparations of these organisms will convert glucose to 2-ketogluconic acid (Wood and Schwerdt, 1953; Claridge and Werkman, 1953). These reactions suggest a pathway for the dissimilation of glucose differing from the glycolytic route. Campbell and Norris (1950) present evidence for the absence of an Embden-Meyerhof scheme in *Pseudomonas aeruginosa* based upon the phosphorus distribution. However, Wood and Schwerdt (1952*a*) have found evidence for alternate routes of carbohydrate oxidation in *Pseudomonas fluorescens*. The present report is concerned with the evidence for the presence of more than one pathway for the oxidation of glucose by *P. aeruginosa*.

MATERIALS AND METHODS

Cells and cell-free extracts of *P. aeruginosa* were grown and prepared as previously reported (Claridge and Werkman, 1953). *Escherichia coli* was grown in a peptonized milk medium for 12 hours (Paege and Schlenk, 1950). Transphosphorylase estimations were made by the manometric method of Colowick and Kalckar (1943). The reduction of diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) was measured in a Beckman spectrophotometer, model DU, at 340 mμ. Aldolase activity was determined by the procedure of Bard and Gunsalus (1950). Respiration and fermentation reactions were followed by use of the standard Warburg manometric technique.

RESULTS

Cell-free extracts of *P. aeruginosa* phosphorylate glucose and gluconate, but not 2-ketogluconate nor other hexoses or pentoses (figure 1). Thus, the presence of a hexokinase and a gluconokinase is indicated. The reduction of diphosphopyridine nucleotide and triphosphopyridine nucleotide in the presence of hexosediphosphate is evidence for the presence of a triosephosphate dehydrogenase. Wood and Schwerdt (1952*b*) indicated that these two coenzymes were not reduced in the presence of this substrate with extracts of *P. fluorescens*. With *P. aeruginosa*, both diphosphopyridine nucleotide and triphosphopyridine nucleotide are reduced with glucose-6-phosphate and fructose-6-phosphate but not with glucose, gluconate, 2-ketogluconate, ribose, or glucose-1-phosphate. Diphosphopyridine nucleotide is reduced in the presence of ribose-5-phosphate, but triphosphopyridine nucleotide is not (figures 2 and 3).

Aldolase activity was present in the cell-free extract, with an optimum at pH 8.3. At this

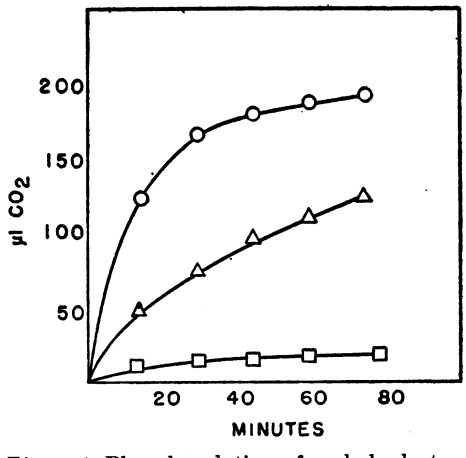

Figure 1. Phosphorylation of carbohydrates by cell-free extract. Each Warburg cup contained 0.5 ml of 0.1 M $NaHCO_3$ buffer, pH 6.65; 10 μM of $MgCl_2$; 60 μM of NaF; 20 μM of substrate; 10 μM of adenosine triphosphate; 0.5 ml of extract; total volume = 2.0 ml; temp = 30.4 C; gas phase, 25 per cent CO_2, 75 per cent N_2. Curve (○) glucose; (△) gluconate; (□) galactose, ribose, 2-ketogluconate, mannose, fructose, xylose, arabinose.

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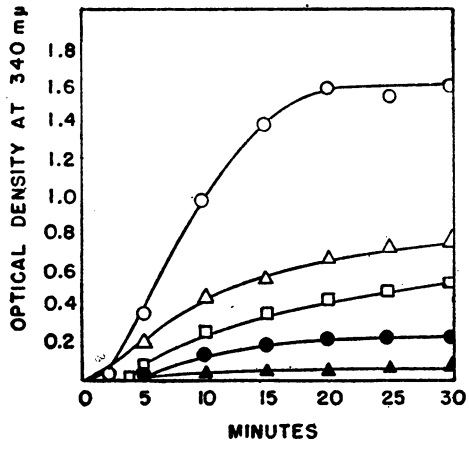

Figure 2. Reduction of diphosphopyridine nucleotide. Each cuvette contained 0.5 ml of 0.2 M phosphate buffer, pH 7.2; 5 μM of substrate; 1.0 ml of diphosphopyridine nucleotide (1 mg per ml); total volume = 3.0 ml; temp = room temperature. Increase in optical density measured at 340 mμ. Curve (○) hexosediphosphate; (△) glucose-6-phosphate; (□) ribose-5-phosphate; (●) fructose-6-phosphate; (▲) glucose-1-phosphate, gluconate, 2-ketogluconate, glucose, ribose, arabinose.

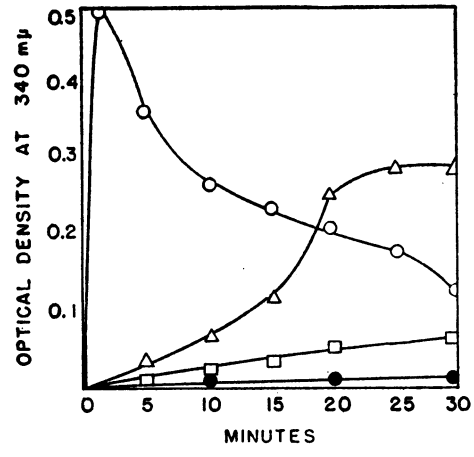

Figure 3. Reduction of triphosphopyridine nucleotide. Each cuvette contained 0.5 ml of 0.2 M phosphate buffer, pH 7.2; 5 μM of substrate; 0.5 ml of triphosphopyridine nucleotide (1 mg per ml); total volume = 3.0 ml; temp = room temperature. Increase in optical density measured at 340 mμ. Curve (○) glucose-6-phosphate; (△) hexosediphosphate; (□) fructose-6-phosphate; (●) glucose-1-phosphate, ribose-5-phosphate, 2-ketogluconate, glucose, gluconate, ribose.

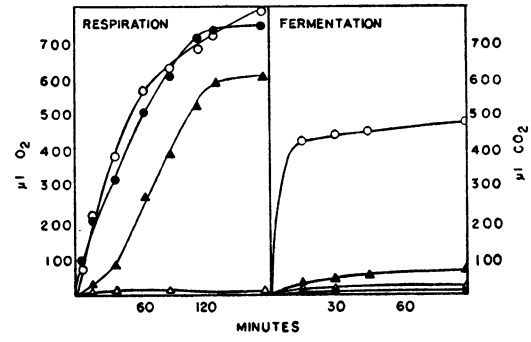

Figure 4. Comparison between fermentation and respiration of *Pseudomonas aeruginosa* and *Escherichia coli*. Fermentation: Each Warburg cup contained 0.5 ml of 0.1 M $NaHCO_3$ buffer; 10 μM of substrate; 100 mg of fresh cells; total volume = 2.0 ml; temp = 30.4 C; gas phase, 25 per cent CO_2, 75 per cent N_2. Respiration: Each Warburg cup contained 0.5 ml of 0.2 M phosphate buffer, pH 7.2; 0.3 ml of 10 N NaOH; 10 μM of substrate; 100 mg of fresh cells, total volume = 2.0 ml; temp = 30.4 C; gas phase, air. Curve (○) glucose; (△) 2-ketogluconate. Shaded symbols—*P. aeruginosa*; clear symbols—*E. coli*.

optimum pH about 37 μg of alkali-labile phosphorus per mg of protein nitrogen per hour were produced. In contrast, Bard and Gunsalus (1950) found that with *Clostridium perfringens* about 1,400 μg were formed under optimum conditions.

Although these results indicate the presence of hexokinase, aldolase, and triosephosphate dehydrogenase, whether or not these enzymes function during growth is not known. Resting cells of *P. aeruginosa* do not appear to possess a glycolytic mechanism. Cells of *P. aeruginosa* were tested aerobically and anaerobically for uptake of O_2 and acid production with glucose and 2-ketogluconate as substrates. The results obtained were compared to the results of an identical experiment with *E. coli* cells (figure 4).

A difference in the metabolism of *P. aeruginosa* and *E. coli* on these two compounds is apparent. Gary and Bard (1952) have reported striking differences in the glucose dissimilatory mechanism of *Bacillus subtilis* which depended upon the type of medium in which the cells had been grown. The patterns of respiration and fermentation of glucose and 2-ketogluconate by *P. aeruginosa* remained the same even when the organism was grown in the presence of a complex nitrogen source.

DISCUSSION

The data presented here indicate the possibility of two types of glucose dissimilatory mechanisms operative in *P. aeruginosa*. Glucose is phosphorylated readily by a cell-free preparation, and thus, an initial reaction in the Embden-Meyerhof scheme is present. Evidence is given also for the presence of triosephosphate dehydrogenase and aldolase. The relatively low activity of this latter enzyme and high pH optimum may be factors in the apparent absence of glycolysis and presence of an active aerobic mechanism for the oxidation of glucose.

The reduction of diphosphopyridine nucleotide and triphosphopyridine nucleotide with glucose-6-phosphate as substrate suggests the presence of the initial reaction of the hexosemonophosphate shunt. The extremely rapid reduction of triphosphopyridine nucleotide indicates the possibility of it being the naturally occurring coenzyme for glucose-6-phosphate dehydrogenation. The reduction of diphosphopyridine nucleotide, but not triphosphopyridine nucleotide with ribose-5-phosphate is in contrast to the similar dehydrogenase found in liver by Glock (1952) which is specific for triphosphopyridine nucleotide only.

The absence of acid formation anaerobically from glucose and 2-ketogluconate suggests the operation of a respiratory or aerobic mechanism during the dissimilation of these two compounds. Glucose and 2-ketogluconate are rapidly metabolized aerobically. Barron and Friedemann (1941) also have reported no evidence for CO_2 production anaerobically from glucose with *P. aeruginosa*.

SUMMARY

Pseudomonas aeruginosa extracts contain hexokinase, gluconokinase, aldolase, and triosephosphate dehydrogenase. However, resting cells do not form acid anaerobically from glucose or 2-ketogluconate, which indicates that the cells have no fermentative capacity. At least part of the enzymes for two types of glucose dissimilation are present in *P. aeruginosa*, but the major functioning pathway remains unknown.

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