Metabolism of Carbohydrates by *Pasteurella pseudotuberculosis*¹

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Cell-free extracts of *Pasteurella pseudotuberculosis* and *P. pestis* catalyzed a rapid and reversible exchange of electrons between pyridine nucleotides. Although the extent of this exchange approximated that promoted by the soluble nicotinamide adenine dinucleotide (phosphate) transhydrogenase of *Pseudomonas fluorescens*, the reaction in the *pasteurellae* was associated with a particulate fraction and was not influenced by adenosine-2'-monophosphate. The ability of *P. pseudotuberculosis* to utilize this system for the maintenance of a large pool of nicotinamide adenine dinucleotide phosphate could not be correlated with significant participation of the Entner-Doudoroff path or catabolic use of the hexose-monophosphate path during metabolism of glucose. As judged by the distribution of radioactivity in metabolic pyruvate, glucose and gluconate were fermented via the Embden-Meyerhof and Entner-Doudoroff paths, respectively. With the exception of hexosediphosphatase, all enzymes of the three paths were detected, although little or no glucokinase or phosphogluconate dehydrase was present unless the organisms were cultured with gluconate. The significance of these findings is discussed with respect to the regulation of carbohydrate metabolism in the *pasteurellae*, related enteric bacteria, and *P. fluorescens*.

Glucose is catabolized by *Pasteurella pestis*, the causative agent of bubonic plague, via the Embden-Meyerhof path (29), whereas gluconate is metabolized by a combination of the Entner-Doudoroff and hexose-monophosphate paths (24). Due to a deficiency of glucose-6-phosphate dehydrogenase (2, 24, 25), *P. pestis* cannot utilize the nonglycolytic paths during growth on hexose. In contrast, this key enzyme is present in *P. pseudotuberculosis* (25), an organism very closely related to *P. pestis* (27). Few studies have been reported on the catabolism of carbohydrates by *P. pseudotuberculosis*; however, the biosynthesis of uncommon sugar compounds by this organism has received intensive study (23, 31).

Cell-free extracts of *P. pestis* and *P. pseudotuberculosis* catalyze a rapid oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH₂) at the expense of nicotinamide adenine dinucleotide (NAD). The resulting large pool of nicotinamide adenine dinucleotide phosphate (NADP) cannot, because of the metabolic block already noted, influence the metabolic fate of glucose-6-phosphate in *P. pestis*. The possibility exists, however, that excess NADP in *P. pseudotuberculosis* might increase the turnover of glucose-6-phosphate dehydrogenase and thus permit hexose catabolism to occur via the nonglycolytic paths (8). The purpose of this study was to define the central paths of carbohydrate metabolism in *P. pseudotuberculosis* and to determine the effect, if any, of the transhydrogenase activity on the participation of these paths.

**MATERIALS AND METHODS**

*Bacteria.* *P. pseudotuberculosis* strain PBI/+ was isolated by Burrows and Bacon (6) during a spontaneous epidemic in laboratory guinea pigs. The properties of virulent (V⁺) and avirulent (V⁻) cells of this strain have already been described (5, 6). *P. pestis* strain EV76, *Pseudomonas fluorescens* strain B-10, and *Escherichia coli* strain B were used in comparative experiments.

*Media and cultivation.* A modification of the salt component of Higuchi and Carlin (15), consisting of 0.025 M K₂HPO₄, 0.01 M citric acid, 0.0025 M MgCl₂, and 0.0001 M FeCl₃, was used in all culture media. Flasks (1-liter) containing 100 ml of this base plus 4 g of CaCl₂·2H₂O were autoclaved and neutralized with NaOH. Then the medium received 1 ml of stock solutions of 0.25 M CaCl₂, 0.25 M Na₂S₂O₃, and 1.0 M carbohydrate (sterilized by filtration). After inoculation with 10⁶ cells per ml, the cultures...
were vigorously aerated at 37°C on a wrist-action shaker, and the organisms were harvested during the early stationary phase by centrifugation at 27,000 × g for 10 min in the cold.

Isotopic experiments. Methods for the isolation and degradation of metabolic pyruvate were similar to those used by Fraenkel and Horecker (12) and Eisenberg and Dobrogosz (10). Bacteria were cultivated with glucose or gluconate, washed once with 0.033 M potassium phosphate buffer (pH 7.2), and then suspended to yield a final concentration of 2 mg (dry weight) per ml in a reaction mixture containing 0.05 M potassium phosphate buffer (pH 7.0) and 0.002 M sodium arsenite. After aerating for 10 min at 37°C, the volume was brought to 5 ml by addition of 0.005 M radioactive glucose or gluconate. Utilization of 0.001 M radioactive glucose 6-phosphate accumulation of pyruvate were complete after further incubation for 1 hr. The incubation mixture was then acidified with 1 ml of 5 N HClO₄ and centrifuged at 27,000 × g for 10 min; the clear supernatant fluid was neutralized by dropwise addition of 10 N KOH. After filtration to remove KClO₄, pyruvate was assayed in a portion of the sample with lactic dehydrogenase, and other radioactive products were determined by chromatography and radioautography.

Essentially all radioactivity was associated with pyruvate, although insignificant amounts of radioactive mannitol (12, 20). Evolved CO₂ was collected in 0.2 ml of 1 N KOH, transferred to a closed vessel, acidified with excess H₂SO₄, and collected in 1.0 ml of 1 N Hyamine hydroxide in methanol. Radioactivity was measured with a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) in Bray's scintillation liquid (3). No more than 0.1 ml of aqueous sample (in 2 ml of methanol) was mixed with scintillation fluid. The same procedures were used to determine ¹⁴C released during metabolism of glucose-1⁻¹⁴C and glucose-6⁻¹⁴C.

Cell-free extracts. Washed whole cells were suspended to a concentration of approximately 10⁶ per ml in cold 0.1 M tri(hydroxymethyl)aminomethane (Tris buffer, pH 7.8). After treatment for 1 min with an ultrasonic probe (Instrumentation Associates, New York, N.Y.), cellular debris was removed by centrifugation at 27,000 × g for 10 min, and the resulting clear extract was dialyzed overnight against cold 0.01 M potassium phosphate buffer plus 0.01 M magnesium phosphate buffer plus 0.001 M EDTA and 0.001 M sodium arsenite.

Enzyme determinations. Most enzymes were determined by methods similar to those used by Mortlock (24) and Vandermark and Wood (32). Unless stated otherwise, a volume of 3 ml was used for spectrophotometric assays. Reactions linked to pyridine nucleotides were followed at 340 μm with a Beckman DU spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) and were corrected, when necessary, for nonspecific oxidase or reductase activity. In all quantitative determinations, one unit of activity is defined as that amount of enzyme necessary to convert 1 μmole of substrate to product per min at 25°C. Results are expressed in terms of specific activity or units of enzyme per mg of protein.

The rate of formation of glucose-6-phosphate was measured in 33.3 mM potassium phosphate buffer (pH 7.2), 3.3 mM MgCl₂, 3.3 mM L-cysteine (pH 7.2), 0.33 mM NADP, and excess yeast glucose-6-phosphate dehydrogenase. Phosphoglucomutase, phosphogluco-

isomerase, and glucokinase were determined in this system by the addition of 3.3 mM glucose-1-phosphate, fructose-6-phosphate, and glucose, plus adenosine triphosphate (ATP), respectively. The basal incubation mixture, without added glucose-6-phosphate dehydrogenase, was used to determine endogenous levels of this enzyme plus phosphogluconate dehydrogenase by the method of Glock and McLean (14); glucokinase was also estimated in the presence of 3.3 mM potassium phosphate and 0.33 mM ATP, and the excess phosphogluconate dehydrogenase present in 1.5 mg of protein obtained from glucose-adapted cells of E. coli.

Glyceraldehyde phosphate was estimated in a reaction mixture consisting of 73 mM Tris buffer (pH 8.0), 10 mM sodium arsenate, 3.3 mM L-cysteine (pH 7.8), 1 mM MgCl₂, and 0.33 mM NADP. This system was used to determine endogenous glyceraldehyde-3-phosphate dehydrogenase after addition of 3 mM glyceraldehyde-3-phosphate. An excess of this enzyme in the presence of 3.3 mM fructose diphosphate or dihydroxyacetone phosphate was used to measure aldolase and triosephosphate isomerase, respectively. The procedure for pyruvate aldolase was similar to that of Mortlock and Rabinowitz (24) except that the reaction mixture was made up of a mixture, without glucose, consisting of 33 mM potassium phosphate buffer (pH 7.2), 3.3 mM reduced glutathione, 3.3 mM MgCl₂, and 0.17 mM reduced nicotinamide adenine dinucleotide (NADH₂). Pyruvate kinase was determined with this reaction system in the presence of excess lactic dehydrogenase, plus 3.3 mM phosphoenolpyruvate, and 3.3 mM adenosine diphosphate; endogenous lactic dehydrogenase was assayed directly with 3.3 mM potassium pyruvate.

Phosphofructokinase was determined by estimation of triose phosphate (28); pyruvate dehydrogenase, phosphate acetyltransferase, and acetate kinase were measured by minor modifications of the procedures outlined by Colowick and Kaplan (7). Methods for determination of enzymes of the Entner-Doudoroff pathway and for qualitative assay of phosphoglycerate kinase, phosphoglucomutase, and phosphopyruvate hydratase are given in Results. The exchange of electrons between pyridine nucleotides was measured by a procedure similar to that used by Eagon (8, 9).

Chromatography. Reactions involving the destruction of ribose 5-phosphate were initiated by the addition of 1 volume of 1 N HClO₄ to the reaction mixture. After removal of protein by centrifugation, the samples were neutralized with 5 N KOH, filtered, and then adjusted to pH 5.5 by dropwise addition of 1 N acetic acid. To each 1 ml of sample was added 1 mg of acid phosphatase and, after incubation for 1 hr at 25°C, the solution was brought to dryness on a steam bath. The free sugars were then extracted in 5 ml of pyridine at 100°C for 10 min, the resulting solutions were filtered, and then pyridine was removed by distillation (22). The residue was dissolved in 10% isopropanol, and samples were chromatographed...
on Whatman no. 1 paper in ascending systems composed of n-butyl alcohol-ethyl alcohol-water (13:8:4, v/v), ethyl acetate-acetic acid-water (3:1:3, v/v), ethyl acetate-pyridine-water (2:1:2, v/v), and water-saturated benzyl alcohol-acetic acid-water (3:1:3, v/v). After chromatography, the sugars were located by spraying with p-anisidine-HCl or ammonical silver nitrate.

Chemical determinations. Pentose and heptulose were assayed by the orcinol reaction (16), and the cysteine-H$_2$SO$_4$ method was used for the determination of hexose (1). Triose phosphate was measured as alkali-labile organic phosphate by addition of 0.5 ml of 1 n NaOH to 0.1 ml of sample; after incubation for 15 min at 25 C, the samples were neutralized and estimated by the method of Fiske and SubbaRow (11). Protein was assayed by the method of Lowry et al. (21).

Reagents. All purified enzymes, coenzymes, sugars and phosphorylated intermediates were purchased from the Sigma Chemical Co. (St. Louis, Mo.), with the exception of 2-keto-3-deoxyphosphogluconate which was received through the courtesy of R. P. Mortlock. Radioactive compounds were obtained from the Volk Radiochemical Co. (Skokie, Ill.).

RESULTS

Preparations of broken V$^+$ cells utilized about six times as much oxygen in the presence of excess NADH$_2$ (0.061 $\mu$ mole of oxygen atoms per mg of protein per min) as was used when NADPH$_2$ served as reductant (0.009 $\mu$ mole per mg of protein per min). The organisms possessed an active NADH$_2$ oxidase system, whereas NADPH$_2$ was slowly oxidized under comparable conditions (Table 1). Although equine cytochrome c was reduced by NADH$_2$ (specific activity = 0.137), NADPH$_2$ did not promote significant reduction of this compound. Since similar results were obtained with analogous artificial electron acceptors, P. pseudotuberculosis probably possessed a respiratory NADH$_2$ dehydrogenase, whereas the existence of a NADH$_2$ dehydrogenase appeared unlikely.

Nevertheless, NADPH$_2$ was rapidly oxidized by NAD, and the reverse reaction could be followed in the presence of endogenous glutathione reductase and added oxidized glutathione. This activity was not influenced by adenosine-2'-monophosphate or other nucleotides tested, and at least one component of the system was removed from solution by centrifugation at 144,000 $\times$ g for 60 min or by passage through a Millipore filter (Table 1). Similar results were obtained from P. pestis and E. coli, although the difference between specific activities in the latter species did not approach that of the Pasteurella species (Table 2). In contrast, the rate of electron exchange catalyzed by the soluble nucleotide-dependent NAD(P) transhydrogenase of P. fluorescens (18) was similar to that noted for the particulate activities in P. pseudotuberculosis and P. pestis. Further study was directed toward defining the significance, if any, of these transhydrogenase systems with regard to the regulation of hexose metabolism.

Radioactive glucose or gluconate was added to suspensions of resting cells, and the distribution of $^{14}$C in metabolic pyruvate was determined. The carboxyl carbon of pyruvate originating from glucose-3,4-$^{14}$C (in glucose-adapted cells) or gluconate-1-$^{14}$C (in gluconate-adapted cells) was heavily labeled (Table 2), which indicated that these compounds were fermented via the

<table>
<thead>
<tr>
<th>Addition to reaction mixture</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal extract</td>
</tr>
<tr>
<td>NADH$_2$</td>
<td>0.074</td>
</tr>
<tr>
<td>NADPH$_2$</td>
<td>0.004</td>
</tr>
<tr>
<td>NADPH$_2$, NAD</td>
<td>0.054</td>
</tr>
<tr>
<td>NADH$_2$, GSSG</td>
<td>0.090</td>
</tr>
<tr>
<td>NADPH$_2$, GSSG, NADP</td>
<td>0.538</td>
</tr>
<tr>
<td>NADH$_2$, GSSG, NADP</td>
<td>0.255</td>
</tr>
</tbody>
</table>

* The reaction mixture contained 100 $\mu$moles of Tris buffer (pH 7.6), 0.4 mg of protein (normal extract) or 0.51 mg of protein (centrifuged extract), excess glutathione reductase, and, where indicated, 0.5 $\mu$ mole of NADH$_2$ or NADPH$_2$, 0.1 $\mu$ mole of NAD or NADP, and 10 $\mu$ moles of GSSG (oxidized glutathione) in a volume of 3.0 ml.

Table 1. Evidence for the presence of NADH$_2$ oxidase and pyridine nucleotide transhydrogenase systems in normal and centrifuged (144,000 $\times$ g, 60 min) extracts of glucose-adapted cells of V$^+$ Pasteurella pseudotuberculosis

Table 2. Determination of NAD(P) transhydrogenase in Pseudomonas fluorescens and analogous activities in Pasteurella pestis and Escherichia coli

<table>
<thead>
<tr>
<th>Addition to reaction mixture</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. fluorescens</td>
</tr>
<tr>
<td>NADH$_2$</td>
<td>0.126</td>
</tr>
<tr>
<td>NADPH$_2$</td>
<td>0.016</td>
</tr>
<tr>
<td>NADPH$_2$, NAD</td>
<td>0.087</td>
</tr>
<tr>
<td>NADH$_2$, GSSG</td>
<td>0.140</td>
</tr>
<tr>
<td>NADPH$_2$, GSSG</td>
<td>0.607</td>
</tr>
<tr>
<td>NADH$_2$, GSSG, NADP</td>
<td>0.385</td>
</tr>
</tbody>
</table>

* The reaction mixture was identical to that described for Table 1.
Embden-Meyerhof and Entner-Doudoroff paths, respectively. Similar results were obtained in control experiments performed with E. coli. P. fluorescens yielded carboxyl-labeled pyruvate after fermentation of both glucose-1-14C and gluconate-1-14C.

To obtain an approximate estimation of the percentage participation of the Embden-Meyerhof, Entner-Doudoroff, and hexose-mono-phosphate paths, 14CO₂ released from glucose-1-14C and glucose-6-14C (in glucose-adapted cells) was collected over periods of 30 min. As judged by differences in rates of evolution (33), a value not exceeding 20% participation was obtained for the hexose-mono-phosphate path in E. coli and V⁺ and V⁻ cells of P. pseudotuberculosis. Glucose- and gluconate-adapted cells released 14CO₂ from glucose-1-14C at similar rates, whereas gluconate was not significantly metabolized by glucose-adapted cells. Gluconate-adapted organisms released 14CO₂ from gluconate-1-14C at a rate which was essentially identical to that obtained with glucose-1-14C.

These results indicated that the ability of P. pseudotuberculosis to maintain NADP in the oxidized state did not, in itself, permit the catabolism of hexose to occur via the nonglycolytic paths. This restriction, however, may have been caused by a metabolic block, perhaps analogous to that in P. pestis. Thus, the presence of the individual enzymes of the nonglycolytic paths was demonstrated by direct or indirect determination.

The velocities obtained during a typical determination of phosphogluconate dehydrogenase and 2-keto-3-deoxyphosphogluconate aldolase in extracts of gluconate-adapted cells are shown in Fig. 1. The aldolase appeared constitutive; specific activities of 0.139 and 0.128 were obtained for extracts of glucose- and gluconate-adapted cells, respectively. In contrast, the dehydrogenase was not detected in glucose-adapted cells, whereas a specific activity of 0.029 was recorded for gluconate-adapted cells. Similarly, little or no gluconokinase was found in glucose-adapted cells; this enzyme, however, yielded a specific activity of 0.304 in extracts of organisms cultivated with gluconate.

The enzymes of the hexose-mono-phosphate pathway did not appear to be under regulatory control. The rate of the formation of glyceraldehyde phosphate from ribose-5-phosphate (via the action of ribulosephosphate-3-epimerase, ribulose-phosphate isomerase, and transketolase) was not altered by cultivation with 5 different sources of energy (Fig. 2). The products formed during the destruction of ribose-5-phosphate by an extract of glucose-adapted cells are given in Table 4. The generation of hexose, coupled with the appearance and disappearance of sedoheptulose as the system approached equilibrium, indicated the presence of transaldolase. Essentially identical velocities were obtained when this experiment was repeated with extracts of gluconate- and xylose-adapted cells. Attempts to demonstrate the presence of phosphoketolase in

### Table 3. Distribution of radioactivity in pyruvate formed during the fermentation of glucose-1-14C, glucose-3,4-14C and gluconate-1-14C

<table>
<thead>
<tr>
<th>Organism</th>
<th>Carbohydrate in growth medium</th>
<th>Fermentation substratea</th>
<th>Percentage of recovered radioactivity</th>
<th>Pyruvate formed (amoles/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total pyruvate</td>
<td>Carboxyl carbon of pyruvatea</td>
</tr>
<tr>
<td>Pasterella pseudotuberculosis</td>
<td>Glucose</td>
<td>Glucose-1-14C</td>
<td>31</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose-3,4-14C</td>
<td>39</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gluconate-1-14C</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose-1-14C</td>
<td>38</td>
<td>8</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Glucose</td>
<td>Glucose-1-14C</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose-3,4-14C</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gluconate-1-14C</td>
<td>34</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose-1-14C</td>
<td>32</td>
<td>7</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>Glucose</td>
<td>Glucose-1-14C</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gluconate-1-14C</td>
<td>13</td>
<td>10</td>
</tr>
</tbody>
</table>

a Initial radioactivity for glucose-1-14C, glucose-3,4-14C, and gluconate-1-14C was 4.1 X 10⁴, 3.9 X 10⁴, and 4.1 X 10⁴ count/min per ml, respectively.

b Measured as 14CO₂ liberated after ceric sulfate degradation.
**P. pseudotuberculosis** by formation of hydroxamates were not successful. Significant hexosediphosphatase could not be detected in extracts of V⁺ or V⁻ cells grown with glucose, gluconate, or glycerol. This determination, based on the generation of NADPH₂ in the presence of added fructose diphosphate, glucosephosphate isomerase, and glucose-6-phosphate dehydrogenase, yielded a specific activity of 0.018 for a similar extract of glycerol-adapted cells of *E. coli*. Specific activities between 0.163 and 0.106 were obtained for glucose-6-phosphate dehydrogenase in extracts of V⁺ and V⁻ cells cultivated with a variety of carbohydrates, and a slightly lower range was recorded for phosphogluconate dehydrogenase.

All glycolytic enzymes were detected and their specific activities, with the exceptions of phosphoglycerate kinase, phosphoglyceromutase, and phosphopyruvate hydratase, are given in Table 5. Velocities obtained during qualitative assays of the latter are shown in Fig. 3. Of special interest was the finding that the specific activity of glucokinase in V⁺ cells was generally about four times greater than that of V⁻ cells.

**DISCUSSION**

The factors which control the metabolic fate of hexose in bacteria are not completely understood. *Salmonella typhimurium* (12) and *E. coli* (10, 13) utilized the Embden-Meyerhof path for fermentation of glucose, whereas the Entner-Doudoroff path was used during growth on gluconate. Evidence for the existence of this catabolic pattern in other organisms has been reported (13, 30). In contrast, many pseudomonads and related bacteria ferment both glucose and gluconate via the Entner-Doudoroff path; these species exhibit a glycolytic block,
GLUCOSE.

But induced markedly that of the erase; analogous phosphate paths but not probably at the level of phosphofructokinase (19). However, the percentage of participation of the Entner-Doudoroff or hexose-monophosphate paths was not increased in mutants of S. typhimurium (12) or E. coli (13) possessing analogous deficiencies of glucosephosphate isomerase; the growth of these mutants on glucose, but not gluconate, was significantly slower than that of the prototrophs. In both cases, glucokinase and phosphogluconate dehydrogenase were markedly induced by gluconate but not by glucose. But Eisenberg and Dobrogosz (10) probably at the level of phosphofructokinase (19). However, the percentage of participation of the Entner-Doudoroff or hexose-monophosphate paths was not increased in mutants of S. typhimurium (12) or E. coli (13) possessing analogous deficiencies of glucosephosphate isomerase; the growth of these mutants on glucose, but not gluconate, was significantly slower than that of the prototrophs. In both cases, glucokinase and phosphogluconate dehydrogenase were markedly induced by gluconate but not by glucose. But Eisenberg and Dobrogosz (10) showed that both substrates induced phosphogluconate dehydrogenase in P. fluorescens and suggested that gluconate, arising from glucose via the action of glucone oxidase, was the actual inducer.

Eagon (8) noted that cell-free extracts of pseudomonads and related nonglycolytic bacteria contained NAD(P) transhydrogenase or respiratory NADP+ dehydrogenase, whereas similar preparations of glycolytic bacteria did not. On this basis, Eagon proposed that the restricted catabolism of hexose via the nonglycolytic paths of the latter species was caused by an inability to maintain a sufficient pool of NADP to permit saturation of glucose-6-phosphate dehydrogenase. In accord with this hypothesis was the finding that methylene blue and pure oxygen promoted a dramatic and probably analogous increase in participation of the hexose-
monophosphate path in erythrocytes (4) and species of Bacillus (26), respectively. Moreover, levels of key enzymes controlling the flow of intermediates and factors regulating the size of the NADP pool may prove to be equally important in determining the catabolic fate of hexose.

It is well established that NADH is generally involved in catabolic reduction, whereas NADPH usually supplies reducing power for biosynthetic reactions (17). The presence of a NADH dehydrogenase in P. pseudotuberculosis is consistent with this concept and was suggested by the determinations given in Table 1. However, the unexpected finding that this organism could rapidly oxidize NADPH in the presence of NAD indicated that the former could indirectly initiate the process of respiration. As a result of this exchange of electrons, it seemed reasonable to assume that the level of NADP in P. pseudotuberculosis might be sufficiently high to favor use of the nonglycolytic pathways during growth on hexose.

The low value obtained for the percentage of participation of the hexose-monophosphate path in P. pseudotuberculosis probably reflects an essentially anaerobic function similar to that described in E. coli (13); nearly identical results were recorded in this study for both organisms. The ability to form glyceraldehyde phosphate (Fig. 2) or hexose, heptulose, or triose phosphates (Table 4) from ribose-5-phosphate did not seem to be influenced by the source of energy used during cultivation. One factor that may have limited the participation of the hexose-monophosphate path in P. pseudotuberculosis was the apparent deficiency of hexosediphosphatase, an enzyme necessary for the complete cyclic oxidation of hexose. The inability to observe hexosediphosphatase probably reflects an instability that is not uncommon for this enzyme, which, according to current knowledge, is also essential for the biosynthesis of hexose and pentose during growth on triose. During the oxidation of glucose it is still possible, even in the absence of hexosediphosphatase, for accumulated triose phosphate to enter the glycolytic path. Fraenkel and Horecker (12) suggested that the capacity of the hexose-monophosphate path in S. typhimurium was constant and limited by the turnover of phosphogluconate dehydrogenase. This may also be true in P. pseudotuberculosis.

The ability of P. pseudotuberculosis to maintain a large pool of NADP did not permit the fermentation of hexose to occur via the Entner-Doudoroff path, although the organism possessed the requisite structural genes. The inability of glucose-adapted cells to utilize the Entner-Doudoroff path can be attributed to a deficiency of phosphogluconate dehydrogenase. However, even when this enzyme was induced, as in glucose-adapted cells, hexose was almost exclusively catabolized by the Embden-Meyerhof path (Table 3). Similar results, previously reported for S. typhimurium (12) and E. coli (10), may reflect a respective high and low affinity of phosphogluconate dehydrogenase and phosphogluconate dehydrogenase for their common substrate (12); the same situation may exist in the case of P. pseudotuberculosis. It should be noted, however, that the soluble NAD(P) transhydrogenase of P. fluorescens can directly reoxidize NADPH generated by the cytoplasmic NADP-linked dehydrogenases. Less efficient reoxidation might be expected in P. pseudotuberculosis, where at least one component of the transhydrogenase activity is particulate in nature and may, therefore, be associated with the cytoplasmic membrane.

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**LITERATURE CITED**


