THE PATHWAYS OF GLUCOSE DISSIMILATION BY *MICROBACTERIUM LACTICUM*[1]

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Received for publication August 8, 1955

Glucose fermentation by lactic acid bacteria occurs by at least two different routes. Homolactic species, the streptococci and homolactobacilli, convert hexoses to lactate by the Embden-Meyerhof pathway. Although the demonstration of glycolytic enzymes and the formation and degradation of glycolytic intermediates by cell-free preparations is incomplete, the conversion of glucose-3,4-C^{14} to carboxyl-labeled lactate by *Lactobacillus casei* (Gibbs *et al.*, 1950) and by *Streptococcus faecalis* (Gibbs *et al.*, 1955) strongly supports this premise. In contrast, the heterolactic organisms (*Leuconostoc* and *Lactobacillus*) ferment glucose via an anaerobic hexose monophosphate pathway. Fermentation of glucose-1-C^{14} and glucose-3,4-C^{14} by *Leuconostoc mesenteroides* (Gunsalus and Gibbs, 1952) and by *Leuconostoc dextranicum* and heterolactobacilli (Gibbs *et al.*, 1955) revealed that carbon 1 of glucose appears as carbon dioxide and carbon 2 as methyl group of ethanol. In addition, enzyme preparations of *L. mesenteroides* were devoid of fructose-1,6-diphosphate aldolase but contained glucose-6-phosphate (G-6-P) dehydrogenase and 6-phosphogluconate (6-PG) dehydrogenase (DeMoss *et al.*, 1951).

Although fermentation of labeled glucose by other *Lactobacteriaceae* such as *Propionibacterium pentosaceum* (Leaver and Wood, 1953) and *Butyribacterium rettgeri* (Pine *et al.*, 1954) leads to labeling of products, which indicates that the Embden-Meyerhof pathway plays a major role in these fermentations, details of the mechanism of hexose fermentation in many of the *Lactobacteriaceae* are almost entirely lacking.

While fermentation appears to be the dominant mechanism of energy generation in these genera, oxidative systems also are present as indicated by the increased growth of *S. faecalis* due to aeration (Seeley and VanDemark, 1951) and by the flavoprotein-linked oxidation of glucose (Seeley and VanDemark, 1951), gluconate, 2-ketogluconate (Sokatch and Gunsalus, *personal communication*), glycerol (Gunsalus and Umbreit, 1945) and pyruvate (O'Kane and Gunsalus, 1948) by *S. faecalis*. This conclusion is further supported by recent demonstrations of an anaerobic hexose monophosphate pathway in *L. mesenteroides* (DeMoss *et al.*, 1953; DeMoss, 1954) and in gluconate-grown *S. faecalis* (Sokatch and Gunsalus, 1954). The oxidation of dicarboxylic acids and propionic acid presumably via cytochromes has been reported in *P. pentosaceum* by Delwiche and Carson (1952).

In order to define further the pathways of carbohydrate metabolism in one of the more aerobic *Lactobacteriaceae*, investigation of the oxidative and glycolytic systems in *Microbacterium lacticum* was undertaken. As shown by Orla-Jensen (1919) this organism is a gram positive, catalase-positive, facultatively aerobic, non-endospore-forming short rod which is generally classified as a member of the *Lactobacteriaceae*, since it produces lactic acid as a major fermentation product. The data to be presented indicate that extracts of this organism contain an intact Embden-Meyerhof scheme and a hexose monophosphate pathway similar to that found in yeast and liver. In addition, a mechanism of pentose phosphate degradation yielding pyruvate but not carbon dioxide was demonstrated.

MATERIALS AND METHODS

Bacteriological. Microbacterium lacticum strain ATCC 8181 used in this study was grown aerobically in a liquid medium containing tryptone, 1 per cent; yeast extract, 1 per cent; K_2HPO_4, 0.5 per cent; and glucose, 0.1 per cent.

[1] This research was supported in part by the grant-in-aid from the National Science Foundation. A preliminary account of this work was presented at the annual meeting of the Society of American Bacteriologists, New York, New York, May 1955.

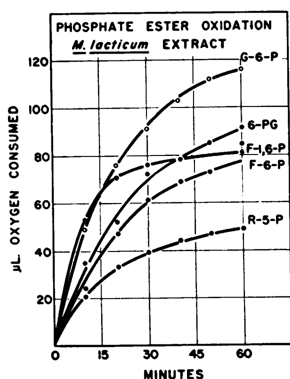

Figure 1. The oxidation of phosphorylated intermediates by a sonic extract of *Microbacterium lacticum*. The Warburg cups contained: protein, approximately 5 mg; the substrates indicated, 5 μM; diphosphopyridine nucleotide (98 per cent purity), 120 μg; M tris buffer, 0.3 ml; pH 7.7; and water to 3 ml. The temperature was 37 C. The endogenous respiration has been subtracted from the data shown.

Five hundred milliliters of medium in Fernbach flasks were inoculated with 1 per cent of an 18-hr culture and incubated on a shaker at 30 C for 18 hr. The cells were removed from the growth medium by centrifugation, washed once with distilled water and resuspended in approximately 1 per cent of the original growth volume. Cell-free extracts were prepared by disrupting the cells in a 200-watt magnetostriction oscillator and removing the debris by centrifugation at 5,000 $\times$ G. The supernatant solution, containing approximately 10 mg of protein per ml, was used as a crude enzyme preparation. In certain experiments, the nucleotides were removed by treating the crude extract with 50 mg per ml of activated carbon ("darco").

Materials. Unless otherwise specified, the substrates employed were obtained and prepared as described previously (Wood and Schwerdt, 1954). Barium-3-phosphoglycerate (PGA) was obtained commercially.

Determinations. Metabolic gas exchanges and acid production (as measured by carbon dioxide release from bicarbonate buffer) were measured with a Warburg respirometer using conventional methods (Umbreit *et al.*, 1949). Protein was estimated by the method of Lowry *et al.* (1951). Aldolase activity was determined by the method of Sibley and Lehninger (1949). Triose phosphate (TP) was estimated either as alkali-labile phosphate (Meyerhof and Lohmann, 1934) or spectrophotometrically with crystalline glyceraldehyde-3-phosphate (G-3-P) dehydrogenase (Cori *et al.*, 1948). Pyruvate was determined colorimetrically by the method of Friedemann and Haugen (1943). Pentose and heptulose were determined by a modification of Mejbaum's procedure as described by Horecker *et al.* (1953). G-6-P and 6-PG were determined by the amount of triphosphopyridine nucleotide (TPN) reduction using purified G-6-P dehydrogenase and 6-PG dehydrogenase according to the method of Horecker and Smyrniotis (1951). Fructose-1,6-diphosphate (F-1,6-P) was estimated spectrophotometrically in the presence of crystalline aldolase and crystalline G-3-P dehydrogenase by the reduction of diphosphopyridine nucleotide (DPN).

Identification of phosphate esters was accomplished by paper chromatography by the procedure of Bandurski and Axelrod (1951). Further identification was obtained by dephosphorylation and paper chromatography of the nonphosphorylated carbohydrates as follows: An aliquot of the reaction mixture was incubated with intestinal phosphatase for 24 hr as described by Schmidt and Thannhauser (1943). The solution was de-ionized with a mixture of "amberlite" IR-45 and "dowex-50," concentrated *in vacuo* and applied to paper strips. Descending chromatograms were developed with water-saturated phenol and the sugars were detected by a combination of the orcinol-spray of Bevenue and Williams (1951) followed by the *p*-aminodimethylaniline-HCl reagent of Koch *et al.* (1951) (Krichevsky, M. I. and Wood, W. A., *unpublished data*).

RESULTS

G-6-P, F-6-P, F-1,6-P, 6-PG and ribose-5-phosphate (R-5-P) were oxidized by crude sonic preparations of *M. lacticum* (figure 1). Carbon dioxide was produced from hexose monophosphate and 6-phosphogluconate but not from R-5-P; i. e., the molar ratio of oxygen consumed to carbon dioxide evolved (R.Q.) was 0.89 for 6-PG and 0.20 for R-5-P. Since the oxygen consumed at the expense of 6-PG and R-5-P approaches 1.0 and 0.5 moles per mole of substrate respectively, the oxidation of 6-PG appears to involve one oxidative decarboxylation and dehydrogenation, whereas that of R-5-P involves one dehydrogenation only.

TABLE 1

The effect of iodoacetate on the aerobic and anaerobic breakdown of phosphate esters

The Warburg flasks contained 1 ml of sonic extract (5 mg protein), 50 mg of substrate, 0.14 μM of diphosphopyridine nucleotide (80 per cent pure). For measurement of oxygen uptake, an additional 1 ml of M tris buffer, pH 7.7, water to 3.0 ml were added. 0.15 ml of 20 per cent KOH was added to the center well. For measurement of acid production, an addition 0.3 ml of 0.26 M sodium bicarbonate, 0.3 ml of M/5 sodium arsenate and water to a 3-ml volume were added. The gas phase was 95 per cent nitrogen–5 per cent carbon dioxide. Sodium iodoacetate (5 μM) was added as indicated.

Substrate	Sodium Iodo-acetate	Oxidation		Fermentation	
		O_2 uptake	Inhibi-tion	CO_2 evo-lution	Inhib-ition
	5×10^{-3} M	μL/hr	%	μL/hr	%
None	−	46		32	
	+	22	48	25	22
Glucose-6-	−	358		202	
phosphate	+	180	50	33	86
Fructose-6-	−	306		165	
diphosphate	+	148	52	24	85
Fructose-1,6-	−	374		220	
diphosphate	+	67	82	63	70
Ribose-5-	−	117		145	
phosphate	+	36	67	38	74
6-Phospho-	−	288		—*	
gluconate	+	178	48		—

* Determinations not made.

The anaerobic dissimilation of G-6-P, F-6-P, F-1,6-P, 6-PG, and R-5-P resulted in acid formation as evidenced by carbon dioxide evolution from bicarbonate buffer. The addition of 5×10^{-3} M sodium iodoacetate inhibited the fermentation of G-6-P, F-6-P to a much greater extent than it did their oxidation (table 1), whereas the inhibition of F-1,6-P and R-5-P degradation was similar under both aerobic and anaerobic conditions. These data suggest that G-6-P and F-6-P are oxidized by a pathway less sensitive to iodoacetate than that involved in their fermentation. F-1,6-P and R-5-P utilization, however, appears to follow the same iodoacetate-sensitive pathway.

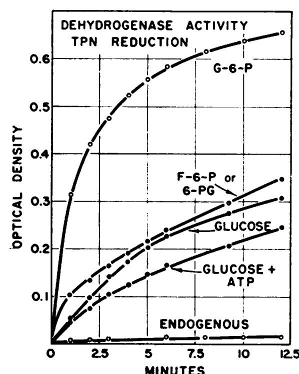

Figure 2. Triphosphopyridine nucleotide reduction by glucose-6-phosphate, 6-phosphogluconate, fructose-6-diphosphate, glucose, and glucose plus adenosine triphosphate. The cuvettes contained: glycylglycine buffer, 150 μM; pH 7.4; sonic extract (approximately 2 mg protein), 0.5 ml; $MgCl_2$, 30 μM; triphosphopyridine nucleotide, 0.5 μM; substrate, 35 μM; and water to a 3-ml volume. 20 μM of adenosine triphosphate was added to the cuvettes as indicated.

Enzymes of the hexose monophosphate pathway. The presence of G-6-P and 6-PG dehydrogenases was demonstrated spectrophotometrically by the reduction of TPN. As shown by the initial reduction, the concentration of G-6-P dehydrogenase was approximately four times that of 6-PG dehydrogenase. These enzymes are highly TPN-specific since with the same hydrogen donors DPN was reduced only slowly. The possibility that an active DPN·H oxidase was preventing DPN·H accumulation was eliminated by running the reactions under oxygen-free conditions in a special anaerobic cuvette. The rate of TPN reduction by F-6-P and by glucose alone and with adenosine triphosphate (ATP) is also shown in figure 2. The presence of a yeast-type 6-phosphogluconate dehydrogenase was also suggested by the transient accumulation of pentose, presumably a mixture of ribulose-5-phosphate and ribose-5-phosphate, during 6-PG oxidation.

A test for the presence of the 6-PG-splitting system discovered in *Pseudomonas saccharophila* by Entner and Doudoroff (1952), which forms pyruvate and glyceraldehyde-3-phosphate via 2-keto-deoxy-6-phosphogluconate, was performed by the procedure of Kovachevich and Wood (1955). The reaction mixture, containing Na 6-PG, 7 μM; $FeSO_4$, 6 μM; and glutathione, 3 μM, was incubated with a crude extract (approxi-

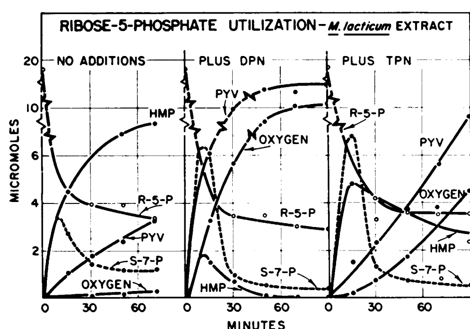

Figure 3. Ribose-5-phosphate degradation by a charcoal-treated sonic extract. Warburg flasks contained approximately: enzyme protein, 2 mg; ribose-5-phosphate, 20 μM; M tris buffer, 0.3 ml; pH 7.7; diphosphopyridine nucleotide or triphosphopyridine nucleotide as indicated, 0.1 μM; and water to a total volume of 1.0 ml. Center well contained 40 per cent KOH, 0.1 ml. The reaction was stopped with 0.1 ml of 50 per cent trichloroacetic acid and the cup contents analyzed as described in Methods.

mately 5 mg of protein). Pyruvate formation could not be detected after incubation for 30 min. Added pyruvate did not disappear under the same conditions (figure 8).

Ribose-5-phosphate degradation. Under anaerobic conditions, ribose-5-phosphate was rapidly degraded with the transient formation of material reacting like sedoheptulose in the orcinol-pentose test (Horecker and Smyrniotis, 1952). The formation and utilization of phosphate esters was followed by paper chromatography of aliquots after phosphatase treatment and de-ionization as described in Materials and Methods. After spraying with the orcinol trichloroacetic acid and *p*-amino-dimethylaniline·HCl reagents, spots corresponding in Rf value and color reaction to ribose, ribulose, sedoheptulose, fructose and glucose were visible. From the intensity of the spots, an initial accumulation and disappearance of ribulose and sedoheptulose, followed by the gradual accumulation of fructose and later glucose, was evident. A similar experiment run under aerobic conditions revealed only small amounts of heptulose and hexose phosphates.

In view of the lack of carbon dioxide formation during ribose-5-phosphate oxidation as noted above, the difference between the results obtained under anaerobic and aerobic conditions appears to result from competition for glyceraldehyde-3-phosphate under aerobic conditions. In order to test this possibility, crude extracts were treated with activated carbon (50 mg/ml) for 5 min to remove DPN and TPN; the carbon was removed by filtration. The aerobic degradation of ribose-5-phosphate by the treated preparation alone and with added DPN or TPN was followed by quantitative determination of pentose, heptulose, triose phosphate, fructose-1,6-diphosphate, hexose phosphates and 6-phosphogluconate. As shown in figure 3, ribose-5-phosphate utilization (15.1 μM) was essentially complete in 15 min. Appreciable amounts of heptulose phosphate, fructose-1,6-diphosphate and triose phosphate were present throughout the incubation period (70 min). During this time, the hexose-monophosphate concentration increased rapidly and pyruvate was formed but at a much slower rate. Oxygen consumption was barely detectable. Under the same conditions, added glucose-6-phosphate and F-1,6-P (source of triose phosphate) were not oxidized. Thus, in the absence of oxidation, hexose monophosphate is a major product of ribose-5-phosphate dissimilation.

When DPN was added to the reaction mixture, the disappearance of ribose-5-phosphate was accompanied by rapid oxygen consumption and pyruvate formation (figure 3). Initially, a large amount of heptulose phosphate and a smaller amount of hexose phosphate were formed; these disappeared rapidly. Thus, in the presence of DPN ribose-5-phosphate is oxidized with the formation of an equivalent amount of pyruvate. The 2-carbon fragment from ribose-5-phosphate was not identified.

In the presence of TPN, the utilization of ribose-5-phosphate was accompanied by an initially rapid increase in heptulose phosphate and hexose phosphate; however, these decreased after incubation for 15 min. A steady-state concentration of triose phosphate and fructose-1,6-diphosphate was present and a moderate rate of pyruvate formation and oxygen consumption was observed.

Glycolytic enzymes. Tests for hexokinase were made by measuring the production of acid during transphosphorylation (evolution of carbon dioxide from bicarbonate, Colowick and Kalckar, 1943), and by coupling glucose phosphorylation with G-6-P oxidation by G-6-P dehydrogenase. With crude extracts, acid production and TPN reduction in the presence of glucose was observed, but the rate was not dependent upon the addition

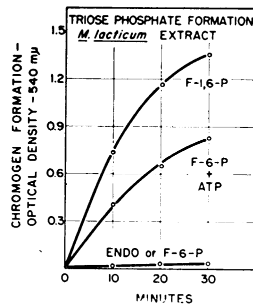

Figure 4. Aldolase and phosphofructokinase activity of a *Microbacterium lacticum* extract. The incubation mixture contained: substrate, 10 μM; enzyme protein, 3 mg; 0.1 M tris buffer, 3.0 ml; pH 8.6; 0.56 M hydrazine, 0.65 ml; and water to a total volume of 7.5 ml. 6.0 μM adenosine triphosphate was added as indicated. One-ml aliquots were added to 2 ml of 10 per cent trichloroacetic acid at the time intervals indicated and analyzed for formation of triose phosphate ("chromogen").

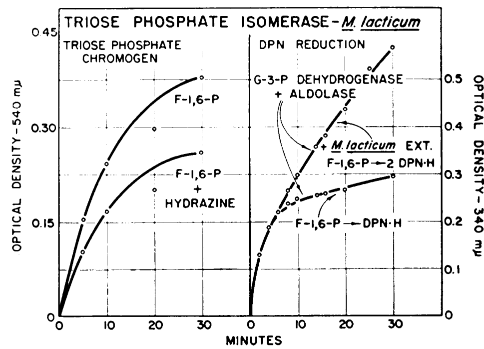

Figure 5 *Figure 6*

Figure 5. Chromogen formation from fructose-1,6-diphosphate in the presence and absence of hydrazine. The incubation conditions and analyses were as indicated in figure 4 except that a charcoal-treated enzyme was used, and hydrazine was omitted as indicated.

Figure 6. Comparative diphosphopyridine nucleotide reduction by fructose-1,6-diphosphate, crystalline aldolase, crystalline glyceraldehyde-3-phosphate dehydrogenase, with and without the sonic preparation of *Microbacterium lacticum*. The cuvettes contained, in a total volume of 3 ml: M tris buffer, 0.8 ml; pH 8.4; glyceraldehyde-3-phosphate dehydrogenase, 0.1 ml (approximately 0.62 mg of protein); μM diphosphopyridine nucleotide, 0.28; fructose-1,6-diphosphate, 0.05 μM; and, where indicated, 0.1 ml of an extract of *M. lacticum*.

of ATP. Following incubation of the extract with glucose and ATP under anaerobic conditions, however, paper chromatography revealed the formation of G-6-P, F-6-P and F-1,6-P.

Evidence for the presence of phosphohexose isomerase was inferred from the observation that ribose-5-phosphate degradation yielded first F-6-P and later G-6-P. This conclusion was further substantiated by the observation that F-6-P evoked TPN reduction (figure 2). Since the F-6-P used was prepared from the crystalline strychnine salt of F-1,6-P, and therefore was free of G-6-P, the reduction of TPN is evidence for the conversion of F-6-P to G-6-P.

The presence of aldolase and phosphofructokinase were demonstrated by the method of Sibley and Lehninger (1949), by which enzymatically formed triose phosphates are fixed as hydrazones and later estimated as a chromogen formed by adding 2,4-dinitrophenylhydrazone and alkali. The addition of hydrazine prevents the conversion of G-3-P to dihydroxyacetone phosphate by triosephosphate isomerase. As shown in figure 4, triose phosphates were rapidly formed from F-1,6-P or F-6-P plus ATP.

Triose phosphate isomerase was demonstrated by a modification of the procedure of Sibley and Lehninger (1949), which utilizes the fact that dihydroxyacetone phosphate contributes 87 per cent of the color developed by equal amounts of both triose phosphates. Thus, in the absence of hydrazine, triose phosphate isomerase converts G-3-P to dihydroxyacetone phosphate, and hence more chromogen is produced in the absence than in the presence of hydrazine. As shown in figure 5, incubation of F-1,6-P with a crude extract in the absence of hydrazine resulted in approximately a 1.5-fold increase in chromogen over that in the presence of hydrazine. Further evidence for isomerase was obtained by showing the reduction of two equivalents of DPN by fructose-1,6-diphosphate in a reaction mixture containing crystalline aldolase and crystalline G-3-P dehydrogenase (each devoid of isomerase) and the crude extracts. As may be calculated from figure 6, with the addition of only crystalline aldolase and crystalline G-3-P dehydrogenase, 0.94 μM DPN·H were formed per 1.0 μM of F-1,6-P added, whereas with the crude enzyme preparation (approximately 2 mg protein) present, 1.82 μM DPN·H accumulated.

G-3-P dehydrogenase was demonstrated by the procedure of Cori *et al.* (1948). Since the rapid oxidation of added DPN·H demonstrated the

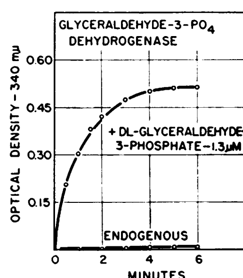

Figure 7. Glyceraldehyde-3-phosphate dehydrogenase content of a sonic extract. The cuvettes contained: 0.04 M sodium pyrophosphate buffer, 2.0 ml; pH 8.5; sodium glutathione, 12 μM; diphosphopyridine nucleotide, 0.5 μM; sodium arsenate, 18 μM, of enzyme protein, approximately 0.1 mg; and 4 μM glyceraldehyde-3-phosphate as indicated. The total volume was 3.0 ml.

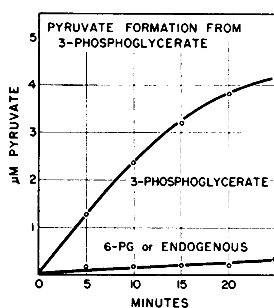

Figure 8. Conversion of 3-phosphoglycerate to pyruvate by a crude extract. The reaction mixture contained: protein, 10 mg; 0.25 M glycylglycine buffer, 2 ml; pH 7.4; substrate and water 50 μM, to a final volume of 6.5 ml. The reaction mixture containing 6-phosphogluconate also contained: 0.56 M hydrazine sulfate (neutralized), 2 ml; of 10^{-2} M sodium arsenate, 2 ml; $FeSO_4$, 6.0 μM; and glutathione, 3 μM. The incubation temperature was 37 C; 1-ml aliquots were removed, the reaction stopped with 0.2 ml of 40 per cent of trichloroacetic acid.

presence of a DPN·H dehydrogenase or a DPN·H oxidase, the initially rapid accumulation of DPN·H without a lag indicates that G-3-P dehydrogenase is present in sufficiently large amounts to mask the competing DPN·H oxidation (figure 7).

The crude extract converted 3-PG to pyruvate, as shown in figure 8. This conversion also was observed with F-1,6-P as the substrate when methylene blue was added as a hydrogen acceptor. Pyruvate was tentatively identified as a toluene and carbonate-soluble 2,4-dinitrophenylhydrazone by the double extraction procedure of Friedemann and Haugen (1943). The conversion of 3-PG to pyruvate indicates the presence of phosphoglyceromutase, and enolase in the sonic extracts.

DISCUSSION

Foregoing data are considered evidence that *M. lacticum* contains both Embden-Meyerhof and hexose-monophosphate pathways for hexose dissimilation. Attempts were not made to demonstrate the presence of a "direct" oxidative system for glucose oxidation via gluconate and ketogluconate. The inability to obtain clear-cut evidence for a hexokinase by conventional methods may reflect its destruction during preparation of the extract or may indicate that the kinase is not of the usual type; e. g., ATP may not be the immediate phosphate donor.

The presence of G-6-P dehydrogenase, 6-PG dehydrogenase and phosphohexose isomerase and the conversion of R-5-P to F-6-P were demonstrated. Thus, the enzymes for the cyclic oxidation of G-6-P, via 6-PG, R-5-P (pentosephosphate isomerase), ribulose-5-phosphate (transketolase), sedoheptulose-7-phosphate (transaldolase), and F-6-P as visualized by Horecker (1953), are present. Extracts of *Pseudomonas fluorescens* (Krichevsky, M. I. and Wood, W. A.; *unpublished data*), *Azotobacter vinelandii* (Mortenson, L. E. and Wilson, P. W.; *personal communication*), and *Acetobacter suboxydans* (Hauge *et al.* 1954) appear to possess a similar array of enzymes. Under aerobic conditions, the oxidation of ribose-5-phosphate solely by this cyclic mechanism should consume one mole of oxygen, yield two moles of carbon dioxide, and one mole of triose phosphate per mole of ribose-5-phosphate completely oxidized. Since the amount of carbon dioxide evolved was small, the observed stoichiometry upon cessation of oxygen consumption approaches R-5-P + $\frac{1}{2}O_2 \rightarrow$ pyruvate + X.

Since F-6-P may be converted to pyruvate by the Embden-Meyerhof pathway, another route from R-5-P to pyruvate involves the formation of F-6-P via transaldolase and transketolase and its conversion to pyruvate by the glycolytic system. In this case, carbon dioxide is not formed, but the ratio of pyruvate formed to ribose-5-phosphate utilized is 1:67.

On the assumption that the only method of degrading ribose-5-phosphate involves its isomerization to ribulose-5-phosphate and cleavage by

transketolase to form S-7-P and G-3-P, the oxidation of G-3-P could prevent the operation of the above cycle, because G-3-P is the acceptor for the transaldolase reaction (Horecker and Smyrniotis, 1955) in which carbon atoms 1, 2 and 3 of sedoheptulose-7-phosphate are transferred to G-3-P to form F-6-P. Since the known reactions of S-7-P, catalyzed by transketolase and transaldolase, require the G-3-P as an acceptor, the removal of G-3-P should cause S-7-P accumulation. S-7-P does not accumulate, however. The data may best be explained by the existence of two reactions for pentose phosphate involving (a) a transfer of the glycolaldehyde moiety to ribose-5-phosphate forming sedoheptulose-7-phosphate and G-3-P and (b) a cleavage yielding a 2-carbon fragment and G-3-P. Whether a new pentose phosphate cleaving enzyme is functioning or whether transketolase both transfers the glycolaldehyde group and forms free glycolaldehyde in a manner analogous to the acetoin and acetaldehyde-forming functions of pyruvate carboxylase (Singer and Pensky, 1952), remains to be ascertained. The fact that both transketolase (Horecker and Smyrniotis, 1953; Racker *et al.*, 1953) and pyruvate carboxylase require the same prosthetic group diphosphothiamin lends credence to this possibility.

SUMMARY

1. Extracts of *Microbacterium lacticum* oxidize glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), fructose-1,6-phosphate (F-1,6-P), 6-phosphogluconate (6-PG), and ribose-5-phosphate (R-5-P). Under anaerobic conditions, F-1,6-P, G-6-P, F-6-P, and R-5-P appear to be fermented by an iodoacetate-sensitive mechanism.

2. Evidence was obtained for the presence of phosphohexose isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, and glyceraldehyde-3-phosphate dehydrogenase. In addition, 3-phosphoglycerate was rapidly converted to pyruvate, indicating the presence of phosphoglyceromutase, enolase and phosphopyruvate transphosphorylase.

3. TPN-linked G-6-P and 6-PG dehydrogenases were demonstrated.

4. Under anaerobic conditions, R-5-P was converted sequentially to ribulose-5-phosphate, sedoheptulose-7-phosphate (S-7-P), F-6-P and G-6-P.

5. Under aerobic conditions R-5-P was oxidized to pyruvate at the expense of S-7-P or hexose monophosphate formation.

6. The data are considered evidence for a new cleavage of ribulose-5-phosphate not involving transketolase.

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