acid decarboxylase activity in the absence of pyridoxal phosphate[3]. Under these conditions oxoglutaric, oxaloacetic and pyruvic acids (mM) induced decarboxylation of *meso*diaminopimelic acid in the Warburg apparatus (Q_{CO_2} of the order of 3.0) but the rate was about 30% of that obtained in the presence of 100 μM pyridoxal phosphate[6]. Transamination by this strain was slow with all three keto acids in the absence of pyridoxal phosphate, but the rate was increased progressively by graded additions of pyridoxal phosphate (μM to 100 μM). The stimulation of decarboxylation by keto acids has not been explained[6].

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SUMMARY

The three stereoisomers of diaminopimelic acid transaminated with one or more of the ketoacids, pyruvic, oxoglutaric or oxaloacetic acid, when incubated with acetone-dried bacteria and pyridoxal phosphate. In certain cases, the DD-isomer was more active than the *meso* form. Transamination of D-lysine also occurred.

REFERENCES

1 D. L. DEWEY, D. S. HOARE AND E. WORK, *Biochem. J.*, 58 (1954) 523.
2 M. ANTIA, D. S. HOARE AND E. WORK, *Biochem. J.*, 65 (1957) 448.
3 J. F. POWELL AND R. E. STRANGE, *Biochem. J.*, 65 (1957) 700.
4 D. L. DEWEY AND E. WORK, *Nature*, 169 (1952) 533.
5 P. MEADOW, D. S. HOARE AND E. WORK, *Biochem. J.*, 66 (1957) 270.
6 P. MEADOW AND E. WORK, *Biochim. Biophys. Acta*, (1958) in the press.
7 L. E. RHULAND, E. WORK, R. F. DENMAN AND D. S. HOARE, *J. Am. Chem. Soc.*, 77 (1955) 4844.

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PENTOSE PHOSPHATE CLEAVAGE BY *LEUCONOSTOC MESENTEROIDES*

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The *Lactobacillus* species ferment pentoses such that the methyl and carboxyl groups of acetate arise from C-1 and C-2 of pentose, respectively, while lactate arises from carbon atoms 3, 4, and 5[1,2]. It has recently been shown that an enzyme preparation purified from *Lactobacillus pentosus* catalyzes a phosphorolytic cleavage of xylulose-5-phosphate, resulting in the production of acetyl P** and triose phosphate[3]. The

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** The following abbreviations are used: acetyl P, acetyl phosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; P_i, inorganic phosphate; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.

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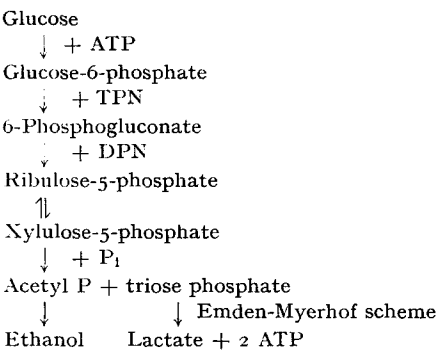
enzyme responsible for this cleavage has been named phosphoketolase. *Leuconostoc mesenteroides*, a heterofermentative organism, produces equimolar quantities of CO_2, ethanol and lactate from glucose. CO_2 arises exclusively from C-1 of glucose, ethanol from carbon atoms 2 and 3, and lactate from carbon atoms 4, 5 and 6[4]. DEMOSS *et al.*[5-8] have shown that extracts of *L. mesenteroides* contain both glucose 6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase. In addition, they were able to demonstrate the formation of a ketopentose phosphate ester and CO_2 from glucose-6-phosphate. The similarity in origin of the two-carbon and three-carbon units from pentose, as described above, suggested a common mechanism of pentose cleavage by *L. mesenteroides* and *L. pentosus*. The results presented below are in accord with a phosphorolytic cleavage of xylulose-5-phosphate by extracts of *L. mesenteroides*. The products of this reaction have been shown to be acetyl P and triose phosphate. In addition, acetyl P can be converted to ethanol by crude extracts of *L. mesenteroides*. These results suggest the fermentation of glucose by *L. mesenteroides* consists of the following series of reactions:



METHODS

The splitting of pentose phosphate was measured by both triose phosphate[9] and acetyl P formation. The latter was determined with acetokinase and ADP.[10]

Starting with ribose-5-phosphate, the reactions involved in determining phosphoketolase activity consisted of:

Ribose-5-phosphate $\rightleftharpoons$ ribulose-5-phosphate $\rightleftharpoons$ xylulose-5-phosphate.
Xylulose-5-phosphate + $^{32}P_i \longrightarrow$ acetyl ^{32}P + triose phosphate.
Acetyl ^{32}P + ADP $\longrightarrow$ acetate + $AT^{32}P$.

Net reaction: Pentose-5-phosphate + ADP + $^{32}P_i \longrightarrow$ acetate + triose phosphate + $AT^{32}P$.

In the presence of an excess of phosphoriboisomerase[11] (isomerase), phosphoketopentoepimerase[12] (epimerase) and acetokinase, the $AT^{32}P$ formed is a direct measure of the phosphoketolase activity. $AT^{32}P$ was adsorbed to charcoal and determined by radioactivity measurement. The assay mixture (0.3 ml) contained 1 μmole of ribose-5-phosphate, 2 μmoles of $MgCl_2$, 0.5 μmole of thiamine pyrophosphate, 2 units of isomerase, 0.16 unit of epimerase, 10 μmoles of potassium buffer, pH 6.0 (30,000 counts/min/μmole), 0.5 μmole of ADP, 0.4 unit of acetokinase and enzyme. Incubation was for 15 min at 38°; 0.1 ml of 1 *N* $HClO_4$ and 0.5 ml of a 30 % suspension of charcoal were then added. The charcoal with the adsorbed nucleotides was washed three times with 2 ml portions of water and finally suspended in 1.0 ml of ethanol–water–ammonia mixture (100:100:2). Aliquots were plated on metal planchets, dried and counted. One unit represents the amount of enzyme which incorporates 1 μmole of P_i into ATP per h as determined above. The specific activity is units per mg of protein[13].

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PREPARATION OF PHOSPHOKETOLASE

The enzyme phosphoketolase was purified from *L. mesenteroides* as follows: Cells were grown in a medium supplemented with 5% tomato juice, as described by DEMOSS *et al.*[5]. After 17 h at 30°, the cells were harvested, washed with 0.02 *M* $NaHCO_3$, and cell-free extracts prepared by grinding 2 g of cells for 10 min with 5 g of alumina A-301 (325 mesh, Aluminum Co. of America). The resulting paste was extracted with 10 ml of 10^{-4} *M* versene and centrifuged for 20 min at 10,000 × *g*. The residue was reextracted with 5 ml more of 10^{-4} *M* versene, and after centrifugation both supernatant fluids were combined (crude extract, 12.5 ml; total units, 225; specific activity, 1.5). The crude extract was treated with 0.05 volumes of 1 *M* $MnCl_2$ and centrifuged after 15 min at 0°. The supernatant fluid ($MnCl_2$ supernatant, 10 ml; total units, 197; specific activity, 4.2) was adjusted to pH 4.0 by the addition of 0.3 ml of 2 *M* ammonium acetate buffer, pH 4.0. 10 min later the suspension was centrifuged and the precipitate discarded. To the supernatant fluid (pH 4.0 supernatant, 10 ml; total units, 175; specific activity, 12.9) 0.05 ml of 0.1 *M* mercaptoethanol and 2.5 ml of acetone (—10°) were added. The suspension was immediately centrifuged at 10,000 × *g*, and to the supernatant fluid (11.5 ml) 10 ml of acetone was added. The resulting precipitate was dissolved in 2.0 ml of water (acetone fraction; total units, 100; specific activity, 30).

RESULTS

With the purified enzyme preparation, the disappearance of ribose 5-phosphate[14] corresponded closely to both acetyl P and triose phosphate formation (Table I, Expt. 1). In a second experiment (Table I, Expt. 2), $^{32}P_i$ fixation coupled to acetokinase, as described above, agreed well with triose phosphate produced. The identification of acetyl P was based on its reactivity in the acetokinase reaction, paper chromatography of the hydroxamate[15], and its reaction in the LIPMANN-TUTTLE test[16].

TABLE I

STOICHIOMETRY OF REACTION

In Expt. 1, the reaction mixture contained 2 μmoles of ribose-5-phosphate, 5 μmoles of $MgCl_2$, 10 μmoles of potassium phosphate buffer, pH 6.0, 0.05 μmole of thiamine pyrophosphate, 1 μmole of mercaptoethanol, 2.5 units of phosphoriboisomerase, 2 units of phosphoketopentoepimerase, and 0.5 mg of Acetone Fraction in a total volume of 0.3 ml. In Expt. 2 the conditions were as given in the text. The above values are expressed in μmoles/ml.

Expt. No.	*Compound measured*	*Time (minutes)*		
		0	*30*	*60*
1	Ribose-5-phosphate	6.70	5.20	3.80
	Acetyl P	0	1.75	2.72
	Triose phosphate	0	1.67	2.38
2	$^{32}P_i$ fixed	0	0.17	0.28
	Triose phosphate	0	0.12	0.21

Evidence has been obtained that xylulose-5-phosphate rather than ribose-5-phosphate or ribulose-5-phosphate is the true substrate of phosphoketolase of *L. mesenteroides* (Table II). Crude extracts readily fixed $^{32}P_i$ into ATP with ribose-5-phosphate as substrate, while with more purified enzyme preparations, additional requirements were necessary. The Acetone Fraction also required the addition of isomerase and epimerase in order to obtain ^{32}P incorporation. Omission of epimerase led to negligible $^{32}P_i$ fixation in the presence of ribose-5-phosphate. Xylulose-5-phosphate addition, however, led to $^{32}P_i$ incorporation in the absence of these two

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enzymes. In addition, fructose-6-phosphate and sedoheptulose-7-phosphate were inactive in the above assay. On the basis of these results, the substrate specificity of phosphoketolase purified from *L. mesenteroides* and *L. pentosus* appear to be identical.

TABLE II

P_i FIXATION BY PURIFIED PHOSPHOKETOLASE (ACETONE FRACTION)

All additions were as described in the text.

	Isomerase	*Epimerase*	^{32}P *fixed* (*μmoles*)
Ribose-5-phosphate	+	+	0.18
Xylulose-5-phosphate	—	—	0.20
Ribose-5-phosphate	+	—	0.02
Sedoheptulose-7-phosphate	—	—	0
Fructose-6-phosphate	—	—	0

Preliminary experiments indicate that extracts of *L. mesenteroides* can convert acetyl P to acetaldehyde in the presence of pyridine nucleotides. This reaction may be similar to the formation of acetaldehyde from acetyl Coenzyme A which occurs in *Clostridium kluyveri*[17].

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SUMMARY

An enzyme preparation has been obtained from *Leuconostoc mesenteroides* which catalyzes the phosphorolytic cleavage of pentose phosphate to yield acetyl phosphate and triose phosphate. Pentose phosphates are cleaved only after conversion to xylulose 5-phosphate. A scheme describing glucose fermentation by *Leuconostoc mesenteroides* is also presented.

REFERENCES

1 J. O. LAMPEN, H. GEST AND J. C. SOWDEN, *J. Bacteriol.*, 61 (1951) 97.
2 D. A. RAPPAPORT, H. A. BARKER AND W. Z. HASSID, *Arch. Biochem. Biophys.*, 31 (1951) 326.
3 E. C. HEATH, J. HURWITZ AND B. L. HORECKER, *J. Am. Chem. Soc.*, 78 (1956) 5443.
4 I. C. GUNSALUS AND M. GIBBS, *J. Biol. Chem.*, 194 (1952) 871.
5 R. D. DEMOSS, R. C. BARD AND I. C. GUNSALUS, *J. Bacteriol.*, 62 (1951) 499.
6 R. D. DEMOSS, I. C. GUNSALUS AND R. C. BARD, *J. Bacteriol.*, 66 (1953) 10.
7 R. D. DEMOSS, *Bacteriol. Proc.*, (1954) 109.
8 R. D. DEMOSS, *J. Cellular Comp. Physiol.*, 41 (1953) Suppl. 207.
9 E. RACKER, *J. Biol. Chem.*, 167 (1947) 843.
10 I. A. ROSE, M. GRUNDBERG-MANAGO, S. R. KOREY AND S. OCHOA, *J. Biol. Chem.*, 211 (1954) 737.
11 J. HURWITZ, A. WEISSBACH, B. L. HORECKER AND P. Z. SMYRNIOTIS, *J. Biol. Chem.*, 218 (1956) 726.
12 J. HURWITZ AND B. L. HORECKER, *J. Biol. Chem.*, 223 (1956) 993.
13 J. BÜCHER, *Biochim. Biophys. Acta*, 1 (1947) 292.
14 W. Z. MEJBAUM, *Z. physiol. Chem.*, 258 (1939) 117.
15 E. R. STADTMAN AND H. A. BARKER, *J. Biol. Chem.*, 184 (1950) 769.
16 F. LIPMANN AND L. C. TUTTLE, *J. Biol. Chem.*, 159 (1945) 21.
17 R. M. BURTON AND E. R. STADTMAN, *J. Biol. Chem.*, 202 (1953) 873.

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