Unit – 1

Carbohydrate catabolic pathways and microbial growth on C 1 compounds

❖ Fermentation of carbohydrates

There are 4 major pathways of carbohydrate breakdown in microorganisms. The sugars are broken down to pyruvate.

1) Glycolysis (EMP)
2) Hexose Monophosphate Pathway (HMP)
3) Entner Duodrof Pathway (ED)
4) Phosphoketolase Pathway

The EMP and HMP occur in both prokaryotes and eukaryotes. The ED and Phosphoketolase pathways occur only in prokaryotes. The organisms of *Enterobacteriaceae* family mainly use EMP and HMP, while *Pseudomonadaceae* use ED pathway.

Fermentations are energy yielding pathways which utilize organic compounds as both electron donors and electron acceptors. Carbohydrates are the main substartes of fermentation.

1) Glycolysis / Embden-Mayerhof-Parnas Pathway (EMP pathway)

The sequence of reactions which converts glucose to pyruvic acid along with the production of ATP is known as Glycolysis. This pathway is also called as EMP pathway after the names of scientists Embden-Mayerhof-Parnas, who have discovered this pathway. It is found in both prokaryotes and eukaryotes.

Under anaerobic condition the pyruvate is converted into either lactic acid or ethanol and is called **Homolactic** or **Alcoholic** fermentation respectively. If the products are more than one such as Acetic acid, Ethanol, Propanol, Propionic acid etc along with lactic acid, then it is called **Heterolactic** fermentation. In aerobic organisms glycolysis leads to TCA cycle and electron transport chain, which releases most energy.

In glycolysis, each glucose molecule forms 2 pyruvate molecules. The breakdown takes place in a series of steps, each catalysed by specific enzyme. Most of the steps of glycolysis are reversible. All enzymes involved in glycolysis are present in cytoplasm i.e. glycolysis occurs in cytoplasm.

Glycolysis is divided into 2 phases—

A) Preparatory phase (Energy utilization phase)
B) Productive phase (Oxidative phase)

A) Preparatory phase (Energy utilization phase)

This has 4 steps

I) Phosphorylation of Glucose: -

Phosphorylation of glucose takes place and Glucose-6-phosphate is formed. The phospahate group is derived from ATP. The reaction is catalysed by *Hexokinase or Glucokinase*.

II) Isomerization of Glucose-6-phosphate to Fructose-6phosphate:-

Glucose-6-phosphate is isomerised to Fructose-6phosphate by *Phosphoglucoisomerase.*

III) Phosphorylation of Fructose-6-phosphate: -

Fructose-6-phosphate is phosphorylated to Fructose-1-6-diphosphate by an enzyme *Phsphofructokinase*. Here one more ATP is utilized.

IV) Cleavage of Fructose-1-6-diphosphate: -

Fructose-1-6-diphosphate splits into two 3 carbon compounds by *Aldolase.* The two compounds formed are Dihydroxy acetone phosphate (DHAP) and 3- Phosphoglyceraldehyde (3 PGAL).

DHAP can be converted into 3 PGAL by an enzyme *Triose phosphate isomerase.* DHAP cannot take part into further reactions of glycolysis. Only 3 PGAL take part into further reactions of glycolysis.

B) Productive phase (Oxidative phase)

This phase has 5 steps

I) Phsphorylation and oxidative dehydrogenation of 3 PGAL: -

This reaction is catalysed by *Glyceraldehyde phosphate dehydrogenase*. The coenzyme involved here is Nicotinamide Adenine Dinucleotide (NAD^+**).** When 3 PGAL is oxidized NAD^+ is reduced. The phosphorylation takes place

on Carbon No. 1. The phosphoric acid (H_3PO_4) provides phosphate. 3 PGAL is converted into 1, 3 Diphosphoglycerate, a high-energy compound.

II) Formation of ATP from 1, 3 Diphosphoglycerate

The high energy phosphate group in 1, 3 Diphosphoglycerate is transfered to ADP resulting in the formation of 3 phosphoglycerate (3 PGA) and ATP by *phosphoglycerate kinase.*

III) Isomerization of 3 PGA into 2 PGA

The 3 Phosphoglyceric acid undergoes rearrangement to form 2 phosphoglyceric acid. The phopsphate group is transfered from 3^{rd} carbon to 2^{nd} carbon by an enzyme *Phosphoglyceromutase.*

IV) Dehydration of 2 PGA

The 2 PGA molecule is dehydrated by *Enolase* to form Phosphoenol pyruvate (PEP) which has a high energy enolic phosphate group.

V) Formation of ATP from PEP

The phosphate group from PEP is transffered to ADP to produce Pyruvic acid and ATP. The reaction is catalysed by *Pyruvate kinase.*

In anaerobic organisms, this pyruvic acid is reduced by NADH + H^+ which is formed in the V^{th} step and lactic acid is formed by *Lactate dehydrogenase.* Thus from one glucose molecule, two lactic acid molecules are formed. There is incomplete degradation of glucose in glycolysis.

In some organisms ethanol and carbon dioxide is produced by *Pyruvate dehydrogenase.*

In aerobic organisms, pyruvate enters into TCA cycle and oxidized completely to CO_2 & H_2O.

★ Energetics of Glycolysis

2 ATP molecules are utilized in preparatory phase of glycolysis and from one 3 PGAL, two ATP molecules are produced and DHAP forms another 3 PGAL, which yields two more ATP molecules.

Thus in glycolysis two ATP molecules are utilized and 4 ATP molecules are produced i.e. there is net gain of only 2 ATP molecules in Glycolysis.

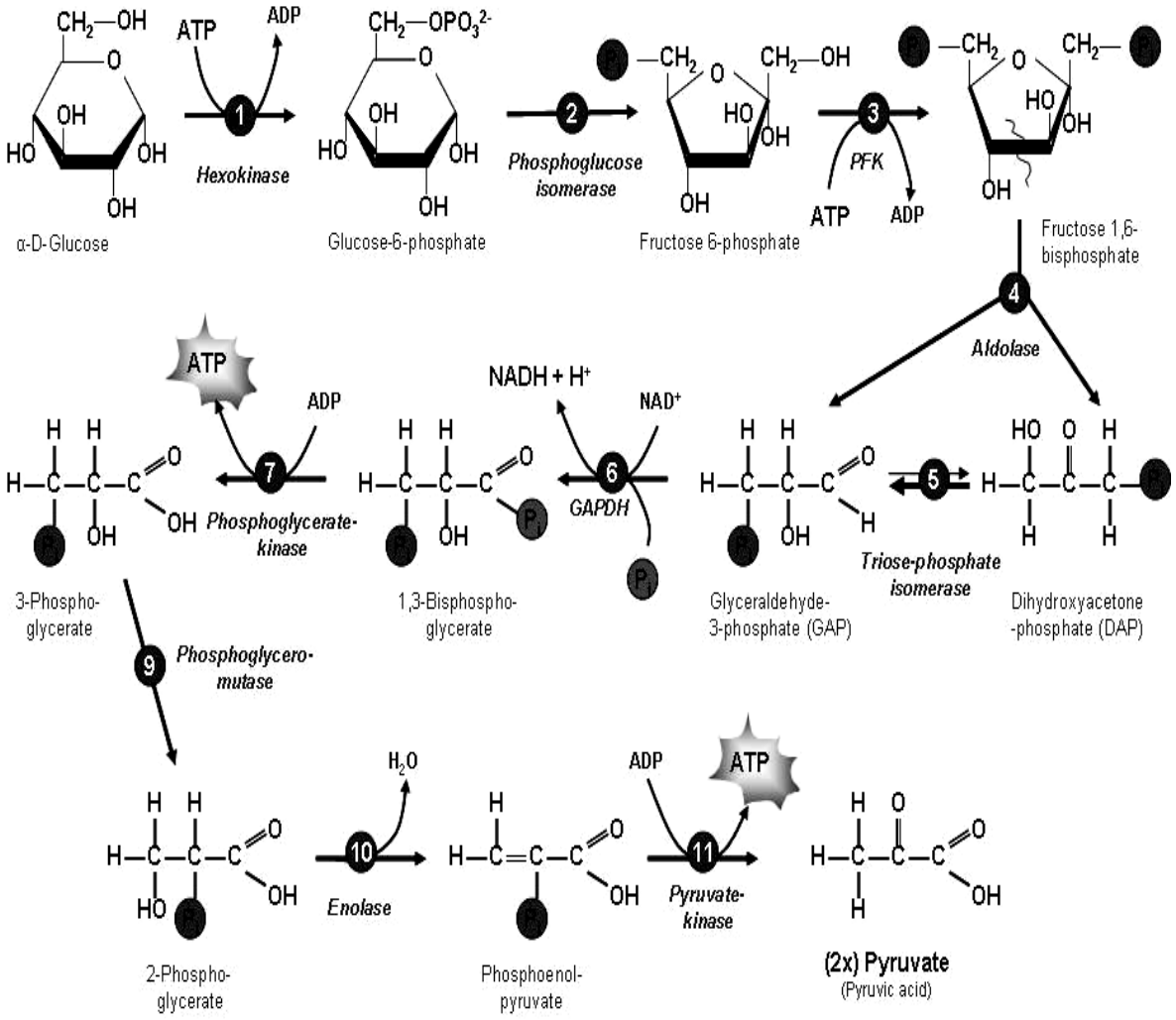
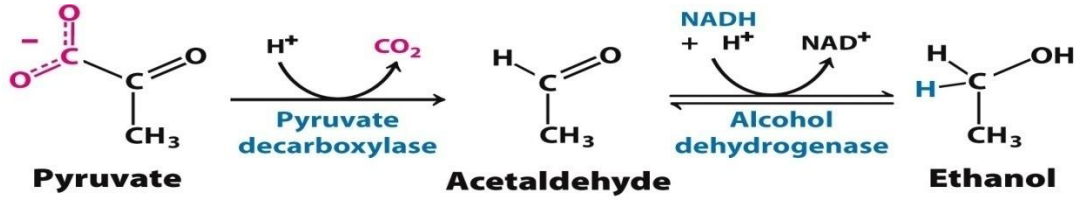


Fig. **Glycolytic pathway**

Alcoholic fermentation

Yeasts ferment glucose to ethanol and carbon dioxide. The fermentation pathway is identical to glycolysis except for terminal step catalysed by lactate dehydrogenase. In alcoholic fermentation, two pyruvate molecules are decarboxylated to acetaldehyde. Then acetaldehyde is reduced to ethanol with $NADH + H^+$ by enzyme ethanol dehydrogenase.



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2**. Hexose Monophosphate Pathway (HMP) /** Hexose monophosphate shunt (H.M.S.) / Pentose phosphate pathway (P.P.P.) / Phosphogluconate pathway (P.G.P.) / Warburg & Dickens's pathway (W&D P.)

Significance: -

1) Primary purpose of this pathway is to generate reducing power in terms of NADPH
2) Secondary function of this pathway is to convert hexoses to pentoses particularly ribose –5 phosphates are required for synthesis of nucleic acids.
3) The third function is oxidative degradation of pentose by conversing them to hexose, which then enters in the glycolysis.
4) This pathway is modified to participate in the formation of glucose from CO_2 in dark reactions of photosynthesis.

The HMP pathway functions in fermentation of several carbohydrates in many microorganisms & is a 'shunt' or 'loop' in the EMP. In this pathway one molecule of glucose-6–phosphate is converted into one molecule of glyceraldehyde, and three molecules of CO_2 are released. The HMP pathway yields only half the energy of the EMP pathway. It is not believed to be a major energy-yielding pathway in microorganism, rather it's function is to provide ribose phosphate for synthesis of nucleotides of RNA and to provide $NADPH_2$ as a source of reducing power.

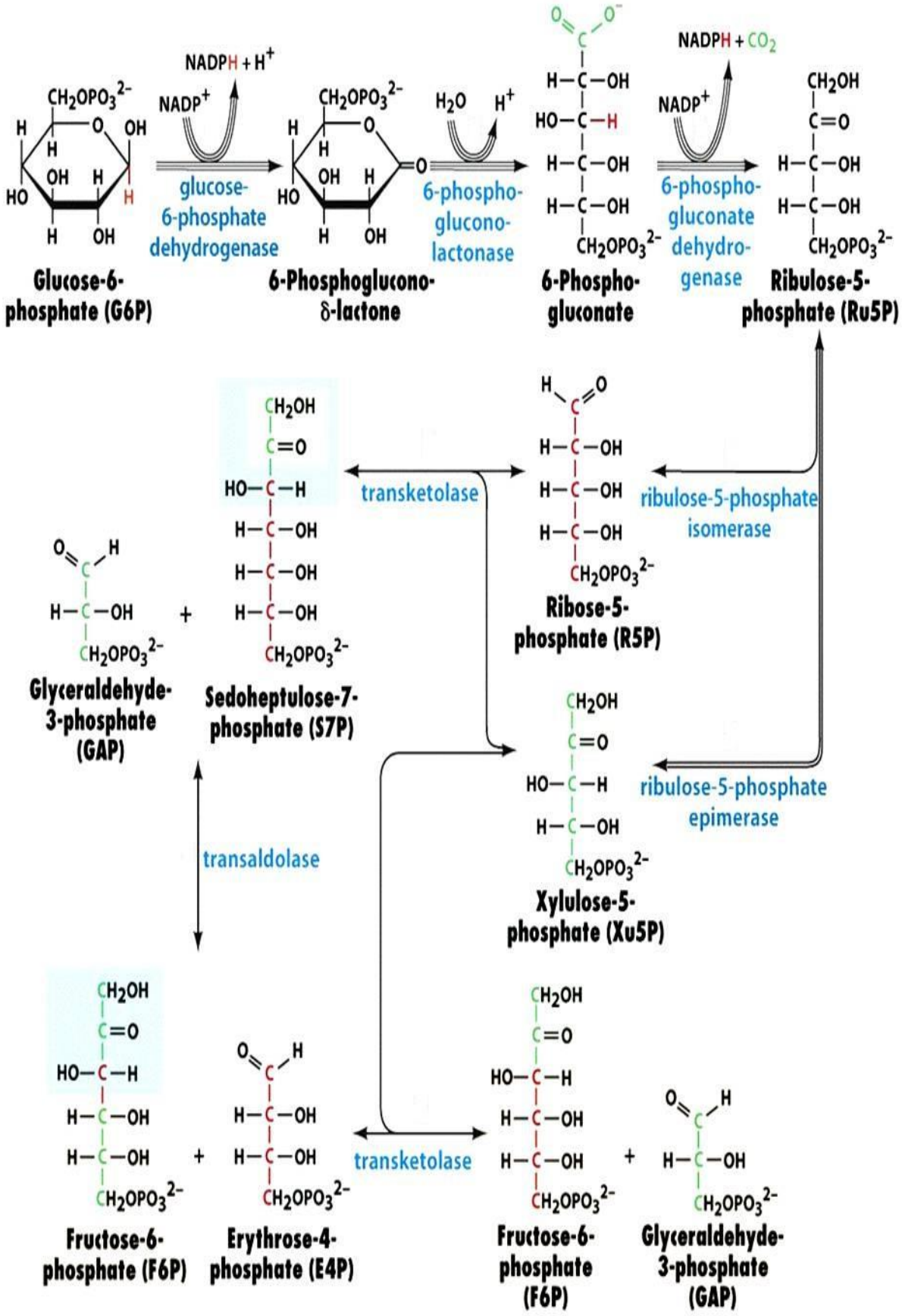
The reduction of NADP takes place at two stages,

1) Oxidation of glucose-6- phosphate to 6- phosphogluconate
2) Oxidation of 6- phosphogluconate to ribose –5- phosphate.

The reactions of HMP pathway are as follows: -

1) Glucose is phosphorylated by a phosphate group obtained from the breakdown of ATP to ADP to form *glucose-6-phpsphate. This* reaction is identical to that of the EMP pathway.

2) Glucose-6-phosphate is oxidized to the *δ*–lactone of phosphogluconic acid by an NADP-linked glucose –6-phosphate dehydrogenase.
3) The *d* lactone of phosphogluconic acid is immediately hydrolysed to 6-phosphogluconic acid by gluconolactonase. (D-glucono–*δ*–lactone hydrolase).
4) 6-phosphogluconic acid is simultaneously decarboxylated & oxidised to form D ribulose –5-phosphate. $NADP^+$ accepts the hydrogens & is reduced to NADPH + $H^{+\cdot}$ The reaction is catalysed by phosphogluconate dehydrogenase. The fourth reaction converts the hexose into a pentose.
5) Two different enzymes act upon the ribulose–5-phosphate. Ribulose phosphate-3-epimerase converts ribulose-5-phosphate to *xylulose–5-phosphate*, while ribose phosphate isomerase converts it to ribose–5-phosphate. Ribose–5-phosphate is a precursor for purine, pyrimidine & aromatic amino acid biosynthesis.
6) Xylulose–5-phosphate (C5) from *sedoheptulose*–7-phosphate (C7) & glyceraldehyde–3-phosphate (C3). Thus two pentose phosphate (C5) molecules react to give a heptose phosphate (C7) & a triose phosphate (C3). The reaction is catalysed by a *transketolase* in the presence of cofactors *thiamine pyrophosphate* (TTP) and Mg^{++}.
7) *Sedoheptulose phosphate* reacts with *glyceraldehyde-3- phosphate* to from *fructose–6- phosphate* and *erythrose-4- phosphate*. This *transaldolation reaction* is catalysed by *transaldolase*. The reaction is reversible. Erythrose –4- phosphate is an important precursor for purine, pyrimidine and aromatic amino acid biosynthesis. Ribose-5- phosphate may also be the acceptor, in which case *octulose-8- phosphate* may be formed.
8) *Erythrose-4- phosphate* accepts a 2-carbon unit from *xylulose –5-phosphate* to form *fructose –6- phosphate* and *gleceraldehyde-6- phosphate*. This reaction is also catalysed by a *transketolase* as in reaction 6. The fructose *–6-phosphate* and the *glyceraldehyde-3- phosphate* of the pathway link up with the EMP pathway. *Fructose –6- phosphate* can be converted to *glucose-6-phosphate* with a *glucose phosphate isomerase (phosphoglucoisomerase)*. Glyceraldehyde-3- phosphate can also follow the reverse EMP pathway to form to *glucose-6- phosphate.*
9) The conversion of *fructose* 1, 6 *diphosphate* to fructose*–6-phosphate* requires an enzymes, hexose diphosphatase, because phosphofructokinase which catalyses reaction 3 in EMP pathway cannot do so in the reverse direction.
10) *Fructose –6- phosphate* gives rise to glucose-6-phosphate by the action of phosphoglucoisomerase, the enzyme which catalyses reaction 2 in the EMP pathway.


Glucose-6-phosphate (G6P)
NADPH + H+
NADP+
glucose-6-phosphate dehydrogenase
6-Phosphoglucono-δ-lactone
H2O
H+
6-phospho-glucono-lactonase
6-Phospho-gluconate
NADPH + CO2
NADP+
6-phospho-gluconate dehydro-genase
Ribulose-5-phosphate (Ru5P)
ribulose-5-phosphate isomerase
Ribose-5-phosphate (R5P)
transketolase
Glyceraldehyde-3-phosphate (GAP)
Sedoheptulose-7-phosphate (S7P)
ribulose-5-phosphate epimerase
Xylulose-5-phosphate (Xu5P)
transaldolase
Fructose-6-phosphate (F6P)
Erythrose-4-phosphate (E4P)
transketolase
Fructose-6-phosphate (F6P)
Glyceraldehyde-3-phosphate (GAP)

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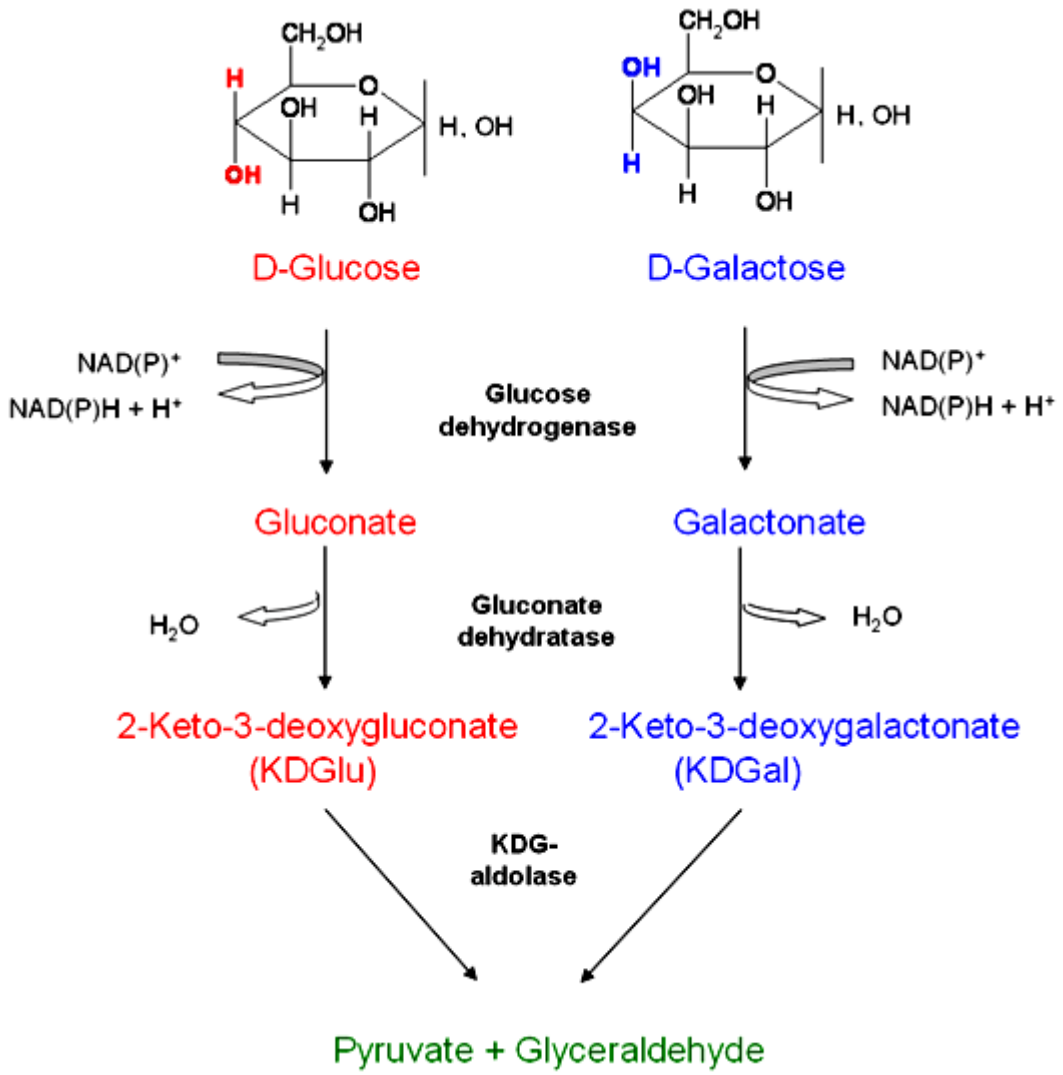
3) The Entner –Duodoroff (ED) pathway

This pathway was discovered by Entner and Duodoroff (1952) in the curse of metabolic studies on *Pseudomonas saccharophilia,* and has been since then fund in many their species of this genus. The first three reactions are identical to those of the HMP pathways. However, not certain whether the enzymes differ in their kinetic characteristics.

i. *Glucose* is phosphorylated to glucose–6-phosphate by ATP, the catalysing enzyme being hexokinase.
ii. Glucose-6 phosphate is oxidized with NADP-linked glucose-6 phosphate dehydrogenase to produce the δ-lactone of phosphogluconic acid (6-phosphoglucono-δ-lactone)
iii. 6-phosphoglucono-δ-lactone is immediately hydrolysed to 6-phosphogluconic acid by gluconolactanase (D-glucono- δ -lactone hydrolase).
iv. 6-phosphogluconic acid is hydrolysed by a phosphogluconate dehydratase to form a ketodeoxy sugar phosphate (2-keto-3-deoxy-6phosphogluconate: KDPG).
v. The ketodeoxy sugar phosphate is cleaved by an aldolase type enzyme (KDPG- aldolase) to pyruvate and glyceraldehyde-3 phosphate.This reaction is very similar to the cleavage of fructose 1, 6-diphosphate in the EMP pathway. KDPG catalyses the enolization of pyruvate.
vi. The metabolites of the ED pathway can lead to other glycolytic pathways:

a) Glucose-6 phosphate can follow the EMP pathway to yield fructose-6 phosphate ⟶ fructose-1,6-diphosphate ⟶dihydroxyacetone phosphate + glyceraldehyde -3 phosphate.
b) 6-phosphogluconic acid can lead to the HMP pathway.
c) Glyceraldehyde-3 phosphate is free to use the EMP pathway to pyruvate.
d) The importance of the pathway lies in the fact that the organisms can produce pentose precursors leading to purine and pyrimidine biosynthesis, as well as the biosynthesis of aromatic amino acids by a reverse HMP pathway. Glyceraldehyde can condense with fructose-6 phosphate to yield erythrose – 4 phosphate and xylulose- 5 phosphate. The reaction being catalysed by a transketolase. Xylulose- 5 phosphate is converted into ribulose- 5 phosphate by ribulose phosphate –3 epimerase. Erythrose-4 phosphate and fructose – 6 phosphate under the catalytic action of an aldolase, form sedoheptulose- 7 phosphate and glyceraldehyde-3 phosphate. A second transketolase catalyses the

formation of xylulose-5 phosphate and ribose –5 phosphate from the above two compounds.



The Entner –Duodoroff (ED) pathway

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(4) Phosphoketolase pathway

The heterofermentative *latobacilli* and the *bifidobacteria* possess the phosphoketolase pathway, which is a variation of the HMP pathway. There are two types of phosphoketolase pathway, the pentose phosphoketolase pathway and the hexose phosphoketolase pathway.

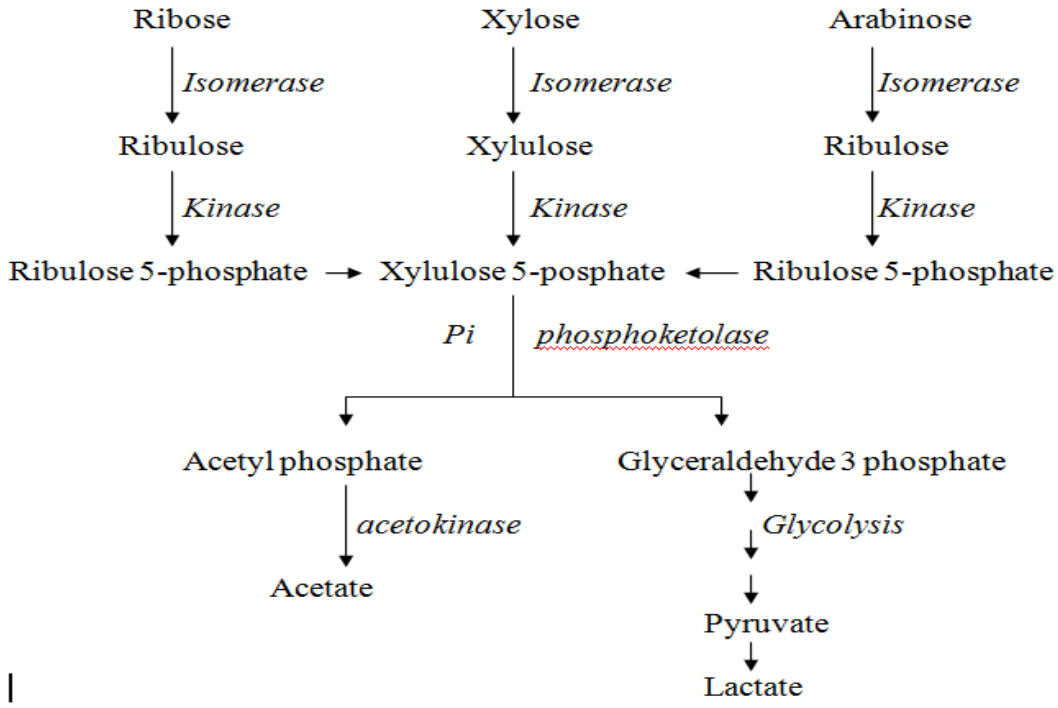
1. **Pentose phosphoketolase pathway**

The carbon source is ribose as well as other pentoses, Ribose-5 phosphate or xylulose-5 phosphate are formed via the HMP pathway. The pentose

phosphoketolase pathway is found in *Leuconostoc mesenteroides* and *Leuconostoc plantarum*, in which the EMP, HMP and ED pathways are absent.

i. *Ribokinase* transfers a phosphate group from ATP to ribose, yielding *ribose-5-phosphate.*
ii. *Ribose-5-phosphate* is isomerised to *ribulose-5-phosphate* by ribose phosphate isomerase.
iii. *Ribulose phosphate-3-epimerase* converts ribulose-5-phosphate to *xylulose –5-phosphate.*
iv. All other pentoses are also converted to xylulos-5-phosphate. Xylose is isomerized to xylulose by *xylose isomerase.*
v. Xylulose is phosphprylated by ATP under the catalytic action of *xylulose kinase* to *xylulose-5-phosphate*.
vi. Arabinose is isomerised to ribulose by arabinose isomerase.
vii. Ribulose is phosphorylated by ATP to *ribulose-5-phosphate* by *ribulokinase.*
viii. *Ribulose-5-phosphate* is converted to *xylulose-5-phosphate* by *ribulosephosphate-4-epimerase.*
ix. Xylulose-5-phosphate plays a key role in the pentose phosphoketolase pathway, because the key enzyme of this pathway, phosphoketolase, reacts only with this compound. The phosphoketolase splits xylulose-5-phosphate into a *acetyl phosphate* and *glyceradehyde*-3-*phosphate*. The reaction requires *thiamine pyrophosphate* (TPP) and *inorganic phosphate* (Pi).
x. *Acetyl phosphate* is converted to *acetate* by *acetokinase.*
xi. *Glyceraldehyde-3-phosphate* is metabolised via the EMP pathway to *pyruvate* and finally *lactate.*

When glucose is the substrate, it is metabolised to ribulose-5-phosphate via the HMP pathway. Further metabolism is by the pentose phosphoketolase pathway.



2. Hexose Phosphoketolase pathways

This pathway is found in genus *bifidobacterium* (*Lactobacillus bifidus*), which lacks *glucose-6-phosphate dehydrogenase* and *fructose diphosphate aldolase*. Because of this, the EMP, HMP, ED or pentose phosphoketolase pathways cannot operate.

The key reaction is the cleavage of *fructose-6-phoshate* into *Erythrose-4-phosphate* and *acetyl phosphate* by *phosphokelase*.

In a reverse HMP pathway involving a *transealdolase* and a *transeketolase*, the compound *xylulose-5-phosphate* is formed. This is split into *glyceraldehyde-3-phosphate* and *acetyl phosphate*, as in the pentose phosphoketolase pathway.

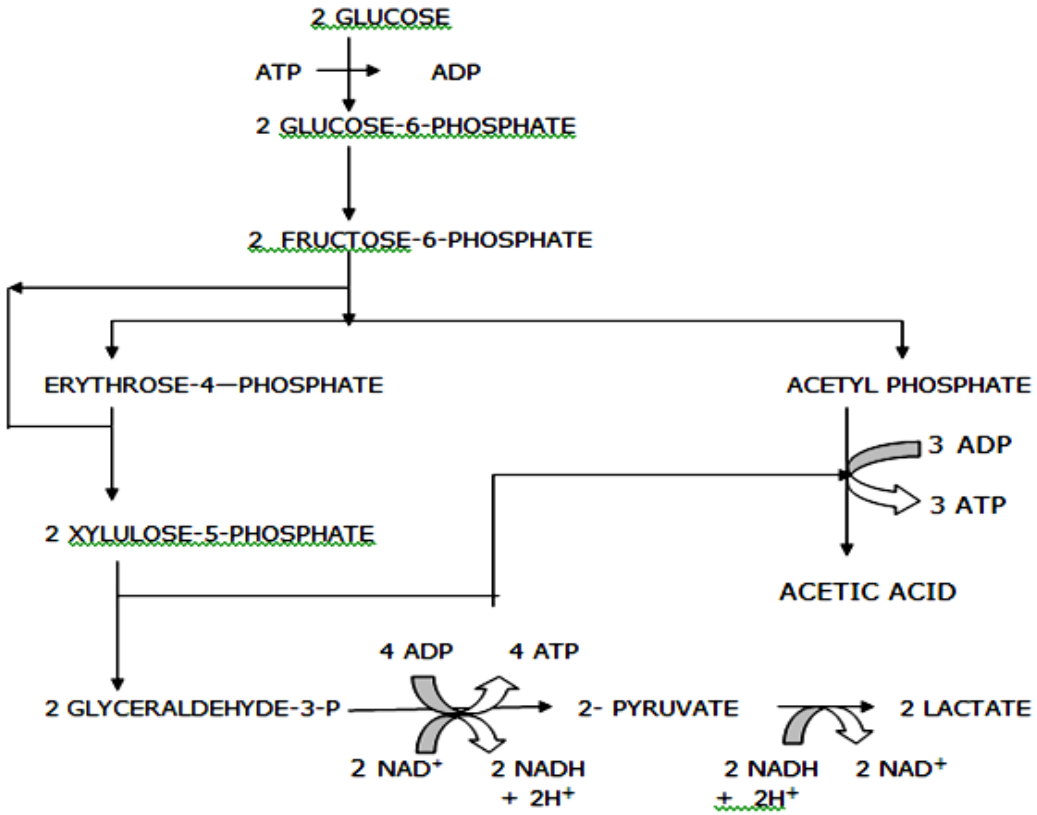


Fig. The hexose phosphoketolase pathway

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❖ Tricarboxylic acid (TCA) cycle / Kreb's cycle / Citric acid cycle

The **Citric acid cycle** – also known as the T**ricarboxylic acid** (**TCA**) **cycle** or the **Kreb's cycle** – is a series of chemical reactions used by all aerobic organisms to generate energy through the oxidation of acetate derived from carbohydrates, fats and proteins into carbon dioxide and chemical energy in the form of adenosine triphosphate (ATP).

The cycle begins with the reaction between acetyl-CoA and the four-carbon oxaloacetate to form six-carbon citric acid. Through the next steps of the cycle, two of the six carbons of the citric acid leave as carbon dioxide to ultimately yield the four carbon product, oxaloacetate, which is used again in the first step of the next cycle. During the eight reactions that take place, for every molecule of acetyl-CoA the cycle produces three NADH and one flavin adenine dinucleotide ($FAD/FADH_2$), along with one molecule of ATP.

The name of this metabolic pathway is derived from citric acid (a type of tricarboxylic acid) that is consumed and then regenerated by this sequence of reactions to complete the cycle. In addition, the cycle consumes acetate (in the form of acetyl-CoA) and water, reduces NAD^+ to NADH, and produces carbon dioxide as a waste byproduct. The NADH generated by the TCA cycle is fed into the oxidative phosphorylation (electron transport) pathway. The net result of these two closely linked pathways is the oxidation of nutrients to produce usable chemical energy in the form of ATP.

In eukaryotic cells, the citric acid cycle occurs in the matrix of the mitochondrion. In prokaryotic cells, such as bacteria which lack mitochondria, the TCA reaction sequence is performed in the cytosol with the proton gradient for ATP production being across the cell's surface (plasma membrane) rather than the inner membrane of the mitochondrion

Pyruvate dehydrogenase complex (PDC)

It is a complex of three enzymes that convert pyruvate into acetyl-CoA by a process called pyruvate decarboxylation. Acetyl-CoA may then be used in the citric acid cycle to carry out cellular respiration, and this complex links the glycolysis metabolic pathway to the citric acid cycle.

Enzyme	Abbrevation	Cofactor(s)

Pyruvate dehydrogenase	E1	TPP (Thiamine pyrophosphate)
Pihydrolipoyl transacetylase	E2	Lipoate Coenzyme A
Dihydrolipoyl dehydrogenase	E3	FAD NAD^+

- **Pyruvate dehydrogenase (E1)**

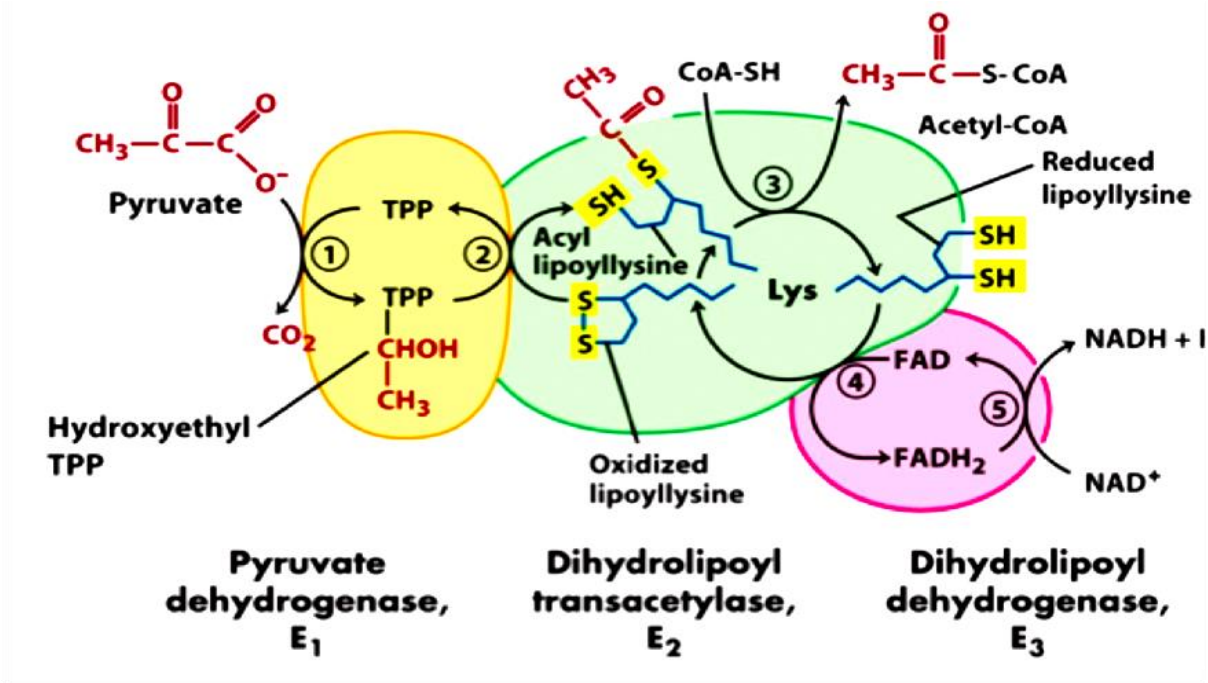
Initially, pyruvate and thiamine pyrophosphate (TPP or vitamin B_1) are bound by pyruvate dehydrogenase subunits. The thiazolium ring of TPP is in a zwitterionic form, and the anionic C2 carbon performs a nucleophilic attack on the C2 (ketone) carbonyl of pyruvate. The resulting hemithioacetal undergoes decarboxylation to produce an acyl anion equivalent. This anion attacks S1 of an oxidized lipoate species that is attached to a lysine residue. In a ring-opening S_N2-like mechanism, S2 is displaced as a sulfide or sulfhydryl moiety. Subsequent collapse of the tetrahedral hemithioacetal ejects thiazole, releasing the TPP cofactor and generating a thioacetate on S1 of lipoate. The E1-catalyzed process is the rate-limiting step of the whole pyruvate dehydrogenase complex.

- **Dihydrolipoyl transacetylase (E2)**

At this point, the lipoate-thioester functionality is translocated into the dihydrolipoyl transacetylase (E2) active site, where a transacylation reaction transfers the acetyl from the "swinging arm" of lipoyl to the thiol of coenzyme A. This produces acetyl-CoA, which is released from the enzyme complex and subsequently enters the citric acid cycle. E2 can also be known as lipoamide reductase-transacetylase.

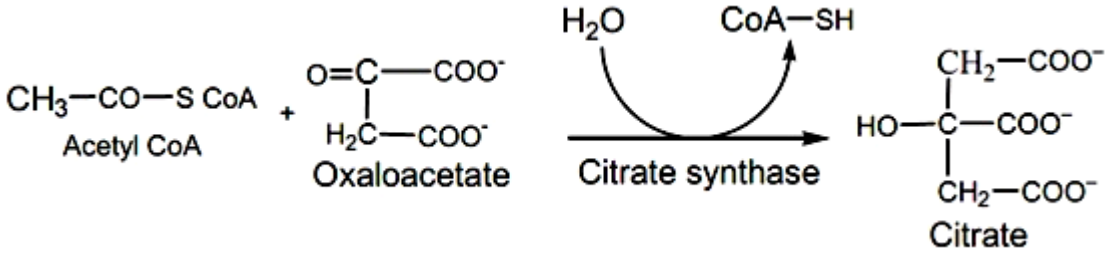
- **Dihydrolipoyl dehydrogenase (E3)**

The dihydrolipoate, still bound to a lysine residue of the complex, then migrates to the dihydrolipoyl dehydrogenase (E3) active site where it undergoes a flavin-mediated oxidation, identical in chemistry to disulfide isomerase. First, FAD oxidizes dihydrolipoate back to its lipoate resting state, producing $FADH_2$. Then, a NAD^+ cofactor oxidizes $FADH_2$ back to its FAD resting state, producing NADH.



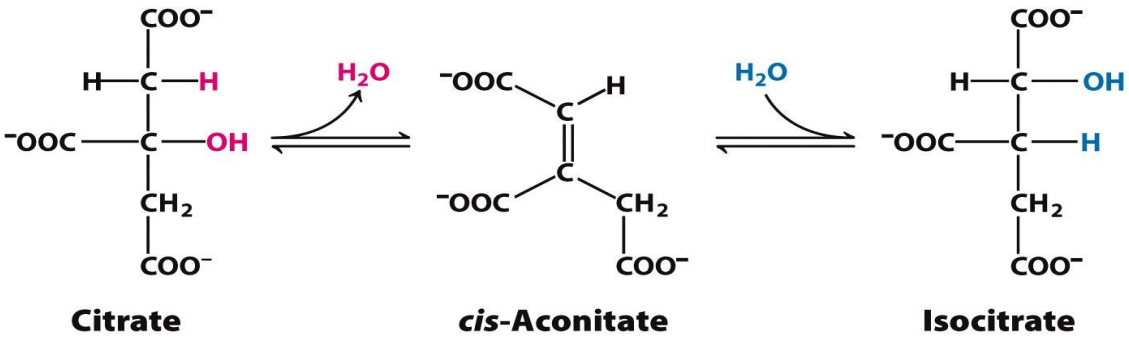
Reaction 1: Citrate Synthase

The first reaction of the citric acid cycle is catalyzed by the enzyme citrate synthase. In this step, oxaloacetate is joined with acetyl-CoA to form citric acid. Once the two molecules are joined, a water molecule attacks the acetyl leading to the release of coenzyme A from the complex.



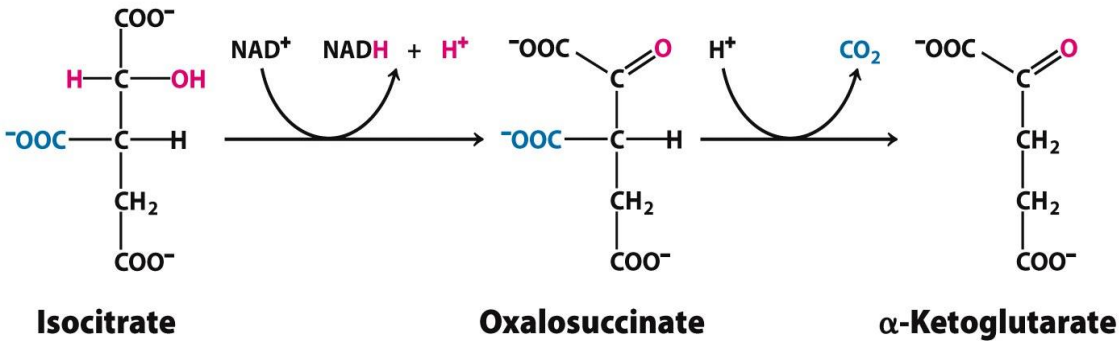
Reaction 2: Acontinase

The next reaction of the citric acid cycle is catalyzed by the enzyme aconitase. In this reaction, a water molecule is removed from the citric acid and then put back on in another location. The overall effect of this conversion is that the –OH group is moved from the 3' to the 4' position on the molecule. This transformation yields the molecule isocitrate.



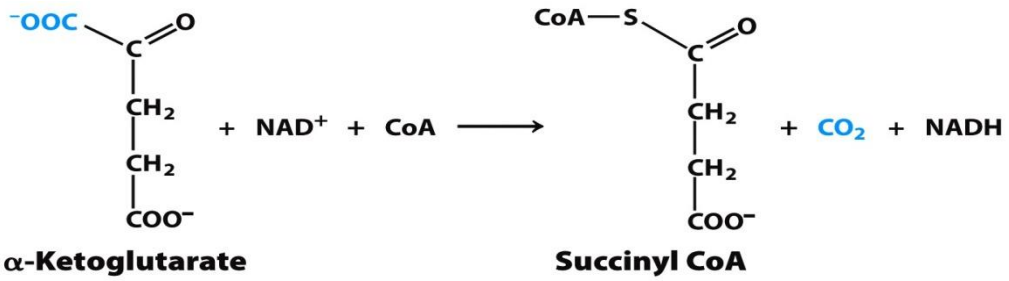
Reaction 3: Isocitrate Dehydrogenase

Two events occur in reaction 3 of the citric acid cycle. In the first reaction, we see our first generation of NADH from NAD. The enzyme isocitrate dehydrogenase catalyzes the oxidation of the –OH group at the 4' position of isocitrate to yield an intermediate which then has a carbon dioxide molecule removed from it to yield α-ketoglutarate.



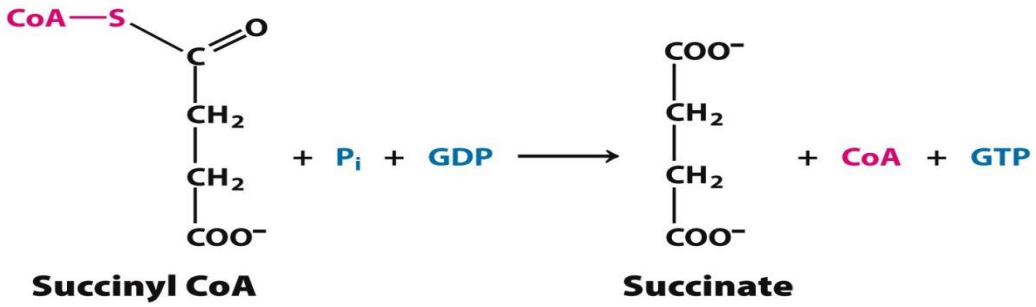
Reaction 4: α-ketoglutarate deydrogenase

In reaction 4 of the citric acid cycle, α-ketoglutarate loses a carbon dioxide molecule and coenzyme A is added in its place. The decarboxylation occurs with the help of NAD, which is converted to NADH. The enzyme that catalyzes this reaction is α-ketoglutarate dehydrogenase. The mechanism of this conversion is very similar to what occurs in the first few steps of pyruvate metabolism. The resulting molecule is called succinyl-CoA.



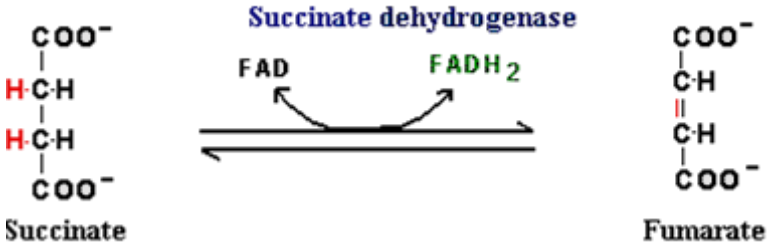
Reaction 5: Succinyl-CoA Synthetase

The enzyme succinyl-CoA synthetase catalyzes the fifth reaction of the citric acid cycle. In this step a molecule of guanosine triphosphate (GTP) is synthesized. GTP is a molecule that is very similar in its structure and energetic properties to ATP and can be used in cells in much the same way. GTP synthesis occurs with the addition of a free phosphate group to a GDP molecule (similar to ATP synthesis from ADP). In this reaction, a free phosphate group first attacks the succinyl-CoA molecule releasing the CoA. After the phosphate is attached to the molecule, it is transferred to the GDP to form GTP. The resulting product is the molecule succinate.



Reaction 6: Succinate Dehydrogenase

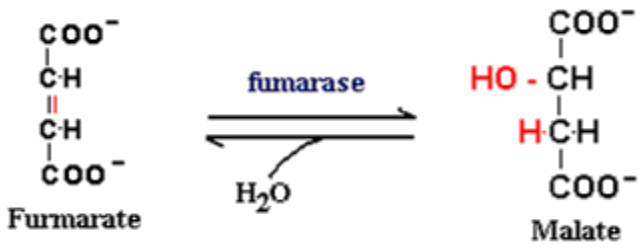
The enzyme succinate dehydrogenase catalyzes the removal of two hydrogens from succinate in the sixth reaction of the citric acid cycle. In the reaction, a molecule of FAD, a coenzyme similar to NAD, is reduced to FADH2 as it takes the hydrogens from succinate. The product of this reaction is fumarate.



FAD, like NAD, is the oxidized form while FADH2 is the reduced form. Although FAD and NAD perform the same oxidative and reductive roles in reactions, FAD and NAD work on different classes of molecules. FAD oxidizes carbon-carbon double and triple bonds while NAD oxidizes mostly carbon-oxygen bonds.

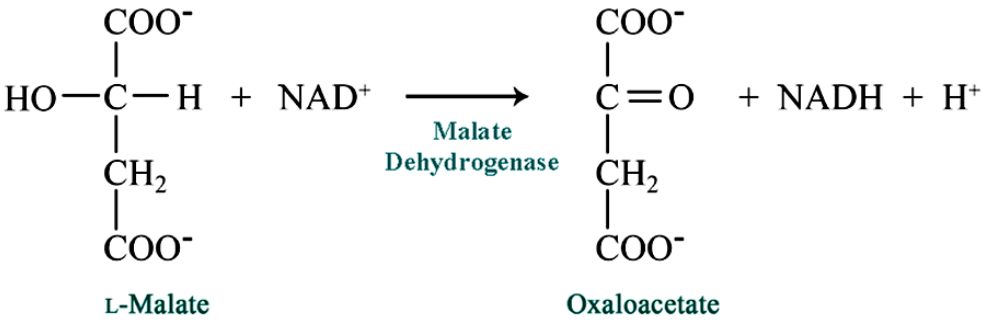
Reaction 7: Fumarase

In this reaction, the enzyme fumarase catalyzes the addition of a water molecule to the fumarate in the form of an –OH group to yield the molecule L-malate.



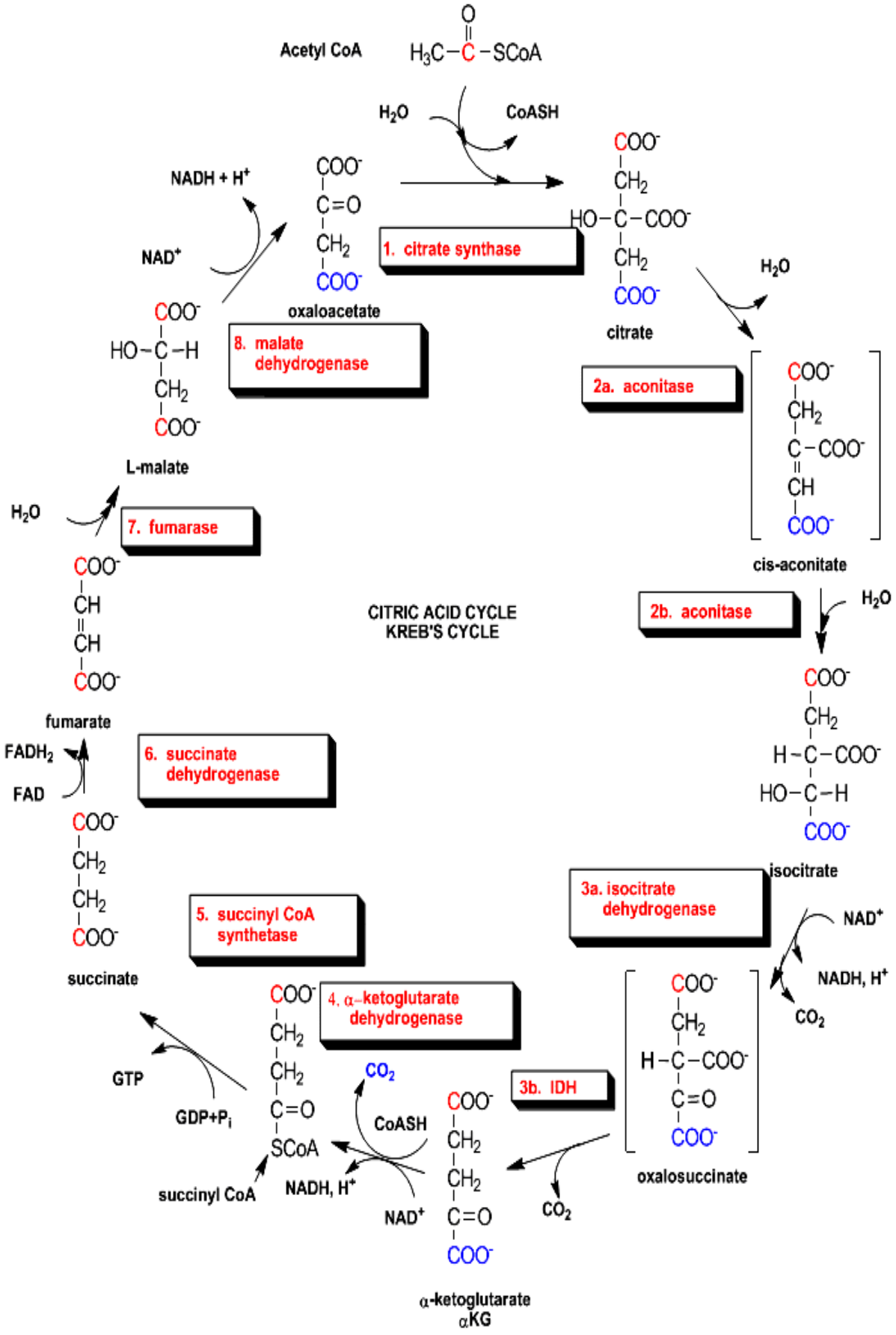
Reaction 8: Malate Dehydrogenase

In the final reaction of the citric acid cycle, oxaloacetate is regenerated by oxidizing L–malate with a molecule of NAD to produce NADH.



- The acetyl-CoA, has been oxidized to two molecules of carbon dioxide.
- Three molecules of NAD were reduced to NADH.
- One molecule of FAD was reduced to FADH2.
- One molecule of GTP (the equivalent of ATP) was produced.

NADH and FADH2 molecules act as electron carriers and are used to generate ATP in the next stage of glucose metabolism, oxidative phosphorylation.



- **Energetics of TCA cycle**

$$Acetyl\text{-}CoA + 2H_2O + 3NAD^+ + Pi + GDP + FAD \Rightarrow 2CO_2 + 3NADH + GTP + CoA\text{-}SH + FADH_2 + 2H^+$$

One turn of the citric acid cycle generates:

- One high-energy phosphate through substrate-level phosphorylation
- Three NADH
- One $FADH_2$

Catabolism of Glucose through Glycolysis and the Krebs Cycle

- Each molecule of Glucose produces two molecules of Pyruvate

$$Glucose + 2NAD^+ + 2ADP + 2Pi \Rightarrow 2Pyruvate + 2NADH + 2H^+ + 2H_2O + 2ATP$$

- Action of *Pyruvate Dehydrogenase* on Pyruvate:

$$Pyruvate + CoA\text{-}SH + NAD^+ \Rightarrow CO_2 + Acetyl\text{-}CoA + NADH$$

- The overall catabolism of Glucose to 2 Pyruvate molecules:

$$Glucose + 2NAD^+ + 2ADP + 2Pi \Rightarrow 2Pyruvate + 2NADH + 2H^+ + 2H_2O + 2ATP$$
$$2Pyruvate + 2CoA\text{-}SH + 2NAD^+ \Rightarrow 2CO_2 + 2Acetyl\text{-}CoA + 2NADH$$

$$Glucose + 4NAD^+ + 2ADP + 2CoA\text{-}SH + 2Pi \Rightarrow 2CO_2 + 2Acetyl\text{-}CoA + 4NADH + 2H^+ + 2H_2O + 2ATP$$

- The GTP formed in the animal *Succinyl-CoA Synthetase* reaction in the Krebs cycle is readily converted to ATP (by *Nucleoside Diphosphokinase*)

$$2Acetyl\text{-}CoA + 4H_2O + 6NAD^+ + 2Pi + 2ADP + 2FAD \Rightarrow 4CO_2 + 6NADH + 2ATP + 2CoA\text{-}SH + 2FADH_2 + 4H^+$$
$$Glucose + 4NAD^+ + 2ADP + 2CoA\text{-}SH + 2Pi \Rightarrow 2CO_2 + 2Acetyl\text{-}CoA + 4NADH + 2H^+ + 2H_2O + 2ATP$$

$$Glucose + 10NAD^+ + 4ADP + 2H_2O + 4Pi + 2FAD \Rightarrow 6CO_2 + 10NADH + 4ATP + 2FADH_2 + 6H^+$$

Yield of ATP

At this point the yield of ATP is 4 moles per mole of Glucose as it passes through the Krebs cycle

- **Regulation of TCA cycle**

The regulation of the TCA cycle is largely determined by product inhibition and substrate availability. If the cycle were permitted to run

unchecked, large amounts of metabolic energy could be wasted in overproduction of reduced coenzyme such as NADH and ATP. The major eventual substrate of the cycle is ADP which gets converted to ATP. A reduced amount of ADP causes accumulation of precursor NADH which in turn can inhibit a number of enzymes. NADH, a product of all dehydrogenases in the TCA cycle with the exception of succinate dehydrogenase, inhibits pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and also citrate synthase. Acetyl-coA inhibits pyruvate dehydrogenase, while succinyl-CoA inhibits alpha-ketoglutarate dehydrogenase and citrate synthase. When tested in vitro with TCA enzymes, ATP inhibits citrate synthase and α-ketoglutarate dehydrogenase; however, ATP levels do not change more than 10% in vivo between rest and vigorous exercise. There is no known allosteric mechanism that can account for large changes in reaction rate from an allosteric effector whose concentration changes less than 10%.[26]

Calcium is used as a regulator. Mitochondrial matrix calcium levels can reach the tens of micromolar levels during cellular activation.[27] It activates pyruvate dehydrogenase phosphatase which in turn activates the pyruvate dehydrogenase complex. Calcium also activates isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.[28] This increases the reaction rate of many of the steps in the cycle, and therefore increases flux throughout the pathway.

Citrate is used for feedback inhibition, as it inhibits phosphofructokinase, an enzyme involved in glycolysis that catalyses formation of fructose 1,6-bisphosphate,a precursor of pyruvate. This prevents a constant high rate of flux when there is an accumulation of citrate and a decrease in substrate for the enzyme.

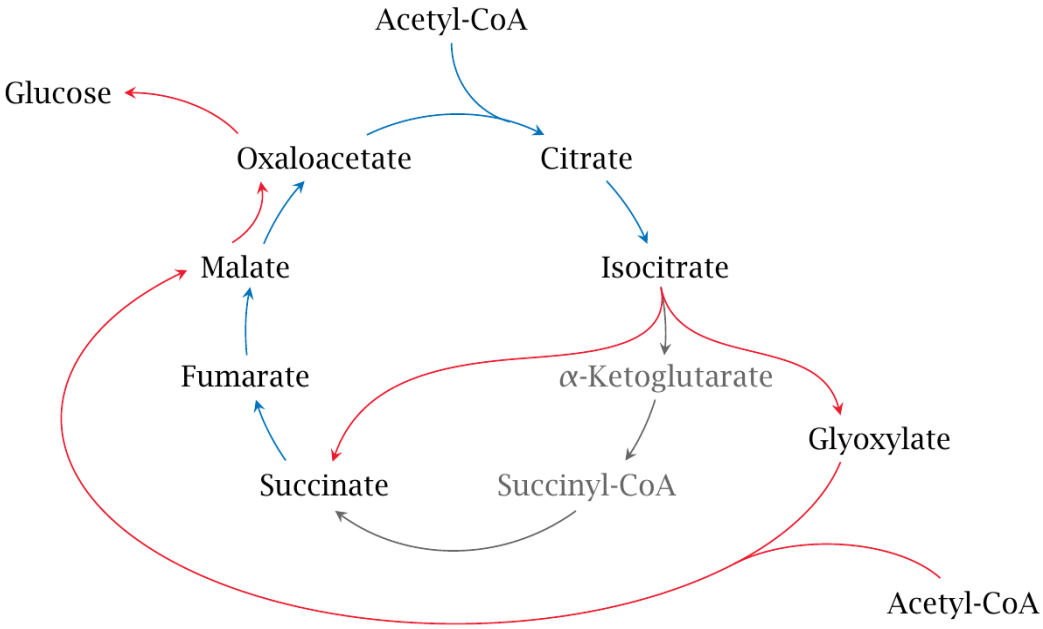
❖ Glyoxylate cycle

The glyoxylate cycle, a variation of the tricarboxylic acid cycle, is an anabolic pathway occurring in plants, bacteria, protists, and fungi. The glyoxylate cycle centers on the conversion of acetyl-CoA to succinate for the synthesis of carbohydrates.[1] In microorganisms, the glyoxylate cycle allows

cells to utilize simple carbon compounds as a carbon source when complex sources such as glucose are not available.[2] The cycle is generally assumed to be absent in animals, with the exception of nematodes at the early stages of embryogenesis. In recent years, however, the detection of malate synthase (MS) and isocitrate lyase (ICL), key enzymes involved in the glyoxylate cycle, in some animal tissue has raised questions regarding the evolutionary relationship of enzymes in bacteria and animals.

Carbon contained in fatty acids cannot be utilized efficiently for gluconeogenesis, since there is no efficient pathway to convert the acetyl-CoA that results from their breakdown into TCA cycle intermediates. Interestingly, however, plants *do* have a straightforward pathway to do just this, namely, the glyoxylate cycle, which is an ancillary road to the TCA cycle.

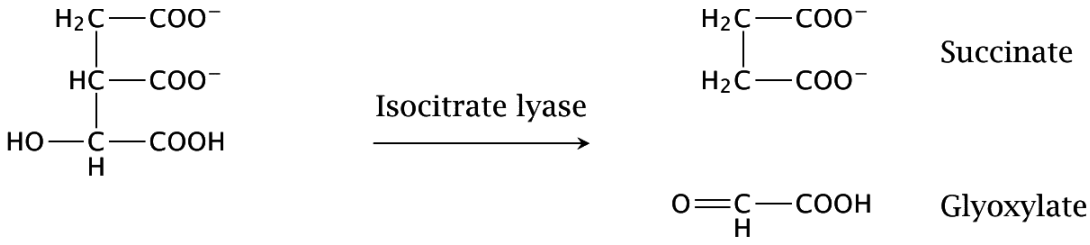
In the glyoxylate cycle, the two decarboxylation steps of the TCA cycle are skipped, and an entry point for a second molecule of acetyl-CoA is created. In this manner, plants are able to use two molecules of acetyl-CoA for the net synthesis of one C_4 TCA cycle intermediate.

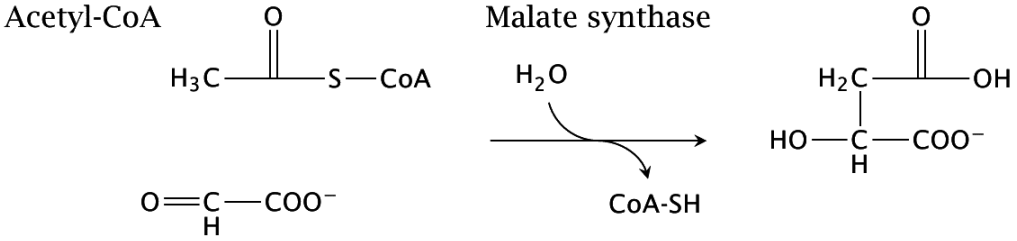


The cycle involves two reactions, both of which are mechanistically similar to citrate synthase:

1. Isocitrate is split into succinate and glyoxylate by isocitrate lyase. Since the isocitrate dehydrogenase and the α-ketoglutarate dehydrogenase reactions are bypassed, the loss of two carbons as CO_2 is avoided; these carbons are retained in the form of glyoxylate.

2. Glyoxylate combines with the second acetyl-CoA molecule to form malate. This reaction is catalyzed by malate synthase, and like the citrate synthase reaction it is pushed forward by the concomitant hydrolysis of coenzyme A.





Many plant seeds are very rich in oil—that is, fat. The glyoxylate cycle enables plant seeds to store metabolic energy and carbon at high density as fat, and to use it for the synthesis of glucose and other carbohydrates during germination.

Similarities with TCA cycle

The glyoxylate cycle utilizes five of the eight enzymes associated with the tricarboxylic acid cycle: citrate synthase, aconitase, succinate dehydrogenase, fumarase, and malate dehydrogenase.

Difference with TCA cycle

The two cycles differ in that in the glyoxylate cycle, isocitrate is converted into glyoxylate and succinate by ICL instead of into α-ketoglutarate. This bypasses the decarboxylation steps that take place in the TCA cycle, allowing simple carbon compounds to be used in the later synthesis of macromolecules, including glucose. Glyoxylate is subsequently combined with acetyl-CoA to produce malate, catalyzed by MS. Malate is also formed in parallel from succinate by the action of succinate dehydrogenase and fumarase.

Role of glyoxylate cycle

In gluconeogenesis

This pathway thus allows cells to obtain energy from fat. To utilize acetate from fat for biosynthesis of carbohydrates, the glyoxylate cycle, whose initial reactions are identical to the TCA cycle, is used.

Cell-wall containing organisms, such as plants, fungi, and bacteria, require very large amounts of carbohydrates during growth for the biosynthesis of complex structural polysaccharides, such as cellulose, glucans, and chitin. In these organisms, in the absence of available carbohydrates (for example, in certain microbial environments or during seed germination in plants), the glyoxylate cycle permits the synthesis of glucose from lipids via acetate generated in fatty acid β-oxidation.

The glyoxylate cycle bypasses the steps in the citric acid cycle where carbon is lost in the form of CO_2. Glyoxylate condenses with acetyl-CoA (a step catalyzed by malate synthase), yielding malate. Both malate and oxaloacetate can be converted into phosphoenolpyruvate, which is the product of phosphoenolpyruvate carboxykinase, the first enzyme in gluconeogenesis. The net result of the glyoxylate cycle is therefore the production of glucose from fatty acids. Succinate generated in the first step can enter into the citric acid cycle to eventually form oxaloacetate.

In plants

In plants the glyoxylate cycle occurs in special peroxisomes which are called glyoxysomes. This cycle allows seeds to use lipids as a source of energy to form the shoot during germination. This cycle allows plants to take in acetate both as a carbon source and as a source of energy.

In fungi

The glyoxylate cycle may serve an entirely different purpose in some species of pathogenic fungi. The levels of the main enzymes of the glyoxylate cycle, ICL and MS, are greatly increased upon contact with a human host.

In vertebrates

Vertebrates were once thought to be unable to perform this cycle because there was no evidence of its two key enzymes, isocitrate lyase and malate synthase. However, some research suggests that this pathway may exist in some, if not all, vertebrates.

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- **Anaplerotic reactions**

Anaplerotic reactions are chemical reactions that form intermediates of a metabolic pathway. Examples of such are found in the citric acid cycle (TCA cycle). In normal function of this cycle for respiration, concentrations of TCA intermediates remain constant; however, many biosynthetic reactions also use these molecules as a substrate. Anaplerosis is the act of replenishing TCA cycle intermediates that have been extracted for biosynthesis (in what are called **cataplerotic reactions**).

The TCA cycle is a hub of metabolism, with central importance in both energy production and biosynthesis. Therefore, it is crucial for the cell to regulate concentrations of TCA cycle metabolites in the mitochondria. Anaplerotic flux must balance cataplerotic flux in order to retain homeostasis of cellular metabolism.

Reactions of anaplerotic metabolism

There are 4 major reactions classed as anaplerotic, and it is estimated that the production of oxaloacetate from pyruvate has the most physiologic importance. The malate is created by PEP carboxylase and malate dehydrogenase in the cytosol. Malate, in the mitochondrial matrix, can be used to make pyruvate (catalyzed by malic enzyme) or oxaloacetic acid, both of which can enter the citric acid cycle.

From	To	Reaction	Notes
Pyruvate	oxaloacetate	pyruvate + HCO_3^- + ATP $\longrightarrow$ oxaloacetate + ADP + P_i + H_2O	This reaction is catalysed by pyruvate carboxylase, an enzyme activated by Acetyl-CoA, indicating a lack of oxaloacetate. It occurs in animal mitochondria. Most important anaplerotic reaction depending on severity, deficiency causes lactic acidosis, severe psychomotor deficiency or death in infancy Pyruvate can also be converted to L-malate, another intermediate, in a similar way.
Aspartate	oxaloacetate	-	This is a reversible reaction forming oxaloacetate from aspartate in a transamination reaction, via aspartate

			transaminase.
Glutamate	α-ketoglutarate	glutamate + NAD^+ + $H_2O \longrightarrow$ NH_4^+ + α-ketoglutarate + NADH + H^+.	This reaction is catalysed by glutamate-dehydrogenase.
β-Oxidation of fatty acids	succinyl-CoA	-	When odd-chain fatty acids are oxidized, one molecule of succinyl-CoA is formed per fatty acid. The final enzyme is methylmalonyl-CoA mutase. Triheptanoin (fat with three heptanoic (C7:0) fatty acids) may be used to treat pyruvate carboxylase deficiency
Adenylo-succinate	fumarate	Adenylo-succinate $\longrightarrow$AMP + fumarate	This reaction is catalysed by adenylosuccinate lyase and occurs in purine synthesis and purine nucleotide cycle. Defect of this enzyme causes psychomotor retardation.

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Catabolism of different carbohydrates

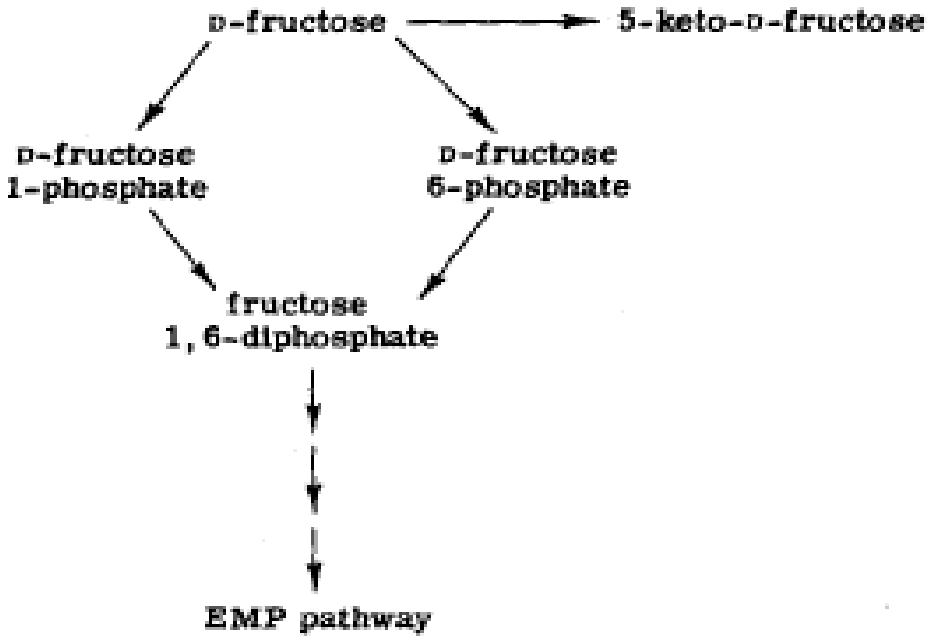
Fructose metabolism

The utilization of fructose is known to occur particularly in *Alcaligenes* and *Aerobacter* but was also found in *Escherichia coli, Zyrmmonas mobilis, Clostridium aceticum*, and *Clostridium thermocellum*. The pathway for this utilization depends on whether the free hexose is supplied extracellularly and must therefore be transported across the cell membrane or whether it is supplied as a product of sucrose utilization inside the cell.

The exogenously supplied fructose is mediated across the cell membrane by a phosphoenolpyruvate: D-fructose 1-phosphotransferase system (ATP:D-fructose 1-phosphotransferase) which is induced by D-fructose and enters the cell as D-fructose 1-phosphate. An inducible enzyme, which has been named D-fructose-l-phosphate kinase (ATP:D-fructose-1-phosphate 6-phosphotransferase) phosphorylates fructose 1-phosphate to fructose 1,6-diphosphate. This enzyme, in contrast to phosphofructokinase is a nonallosteric enzyme and is activated by potassium and ammonium ions.

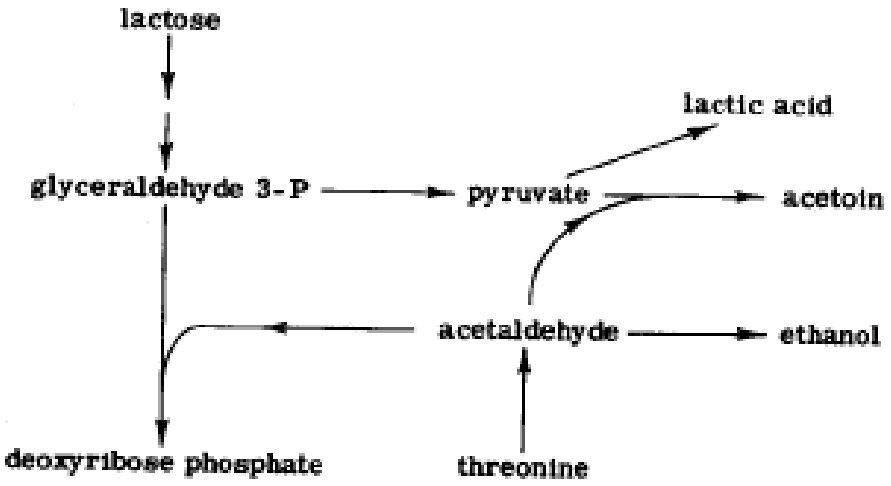
The second pathway is mediated by a specific sucrose-induced D-fructo-kinase (ATP:D-fructose 6-phosphotransferase and phosphorylates D-fructose to fructose 6-phosphate. Both pathways continue via the EMP pathway.

A third pathway exists in *Acetobacter cerinus* , a strictly aerobic organism. The oxidation of D-fructose to 5-keto-D-fructose. This reaction is catalyzed by a NADPH-dependent dicarbonylhexose reductase (D-fructose:$NADP^+$ 5-oxidore-ductase, which is extremely substrate specific and strongly favors the reduction of 5-keto-D-fructose at equilibrium.



Lactose Metabolism

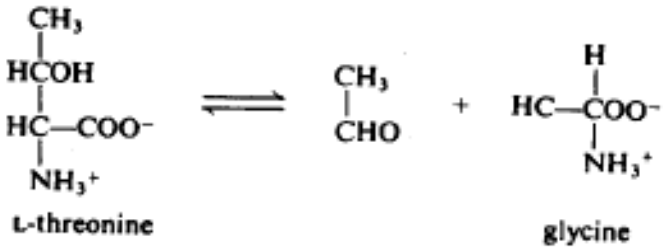
Lactose is very readily metabolized to galactose and glucose by Enterobacteriaceae and Group N streptococci. It is used as one of the main classification criteria, together with genetics, because of the importance of the *lac* operon. Glucose is further metabolized via the EMP pathway.



Scheme 5.4. Lactose metabolism of Group N streptococci.

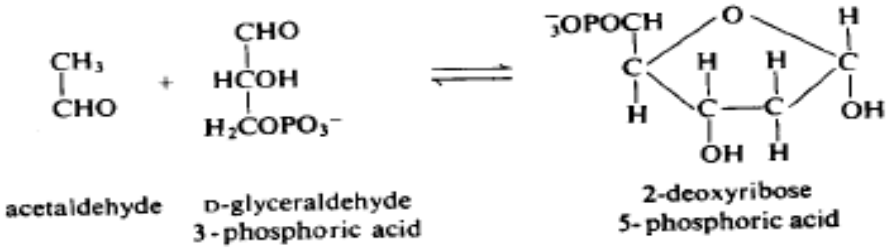
The utilization of lactose by Group N streptococci, however, has been found to differ from that by the Enterobacteriaceae. These bacteria, namely *Streptococcus diacetilactis, S. lactis,* and *S. cremoris,* grow on lactose and

casein hydrolyzate. They utilize threonine with the formation of glycine and acetaldehyde. The enzyme catalyzing the utilization of threonine is threonine aldolase (L-threonine acetaldehyde-lyase) and pyridoxal phosphate is required as cofactor. Acetaldehyde itself can be metabolized further via three different paths, depending on the availability.

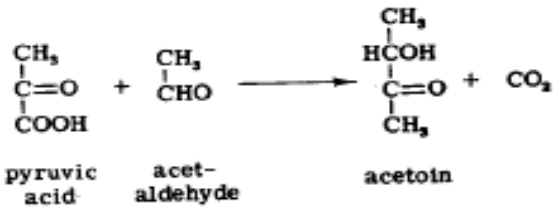


of three enzymes as well as the utilization of lactose via the EMP pathway:

1. Acetaldehyde and glyceraldehyde 3-phosphate are cleaved under the catalytic action of deoxyriboaldolase (2-deoxy-D-ribose-5-phosphate acetaldehyde-lyase, EC 4.1.2.4) to form deoxyribose phosphate:

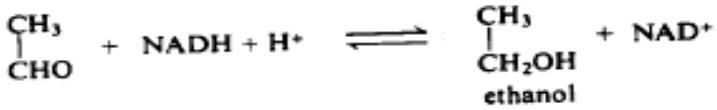


2. The Group N streptococci also possess an enzyme that cleaves pyruvate with acetaldehyde with the formation of acetoin:



This enzyme has been called "acetoin synthetase" by Lees and Jago (personal communication).

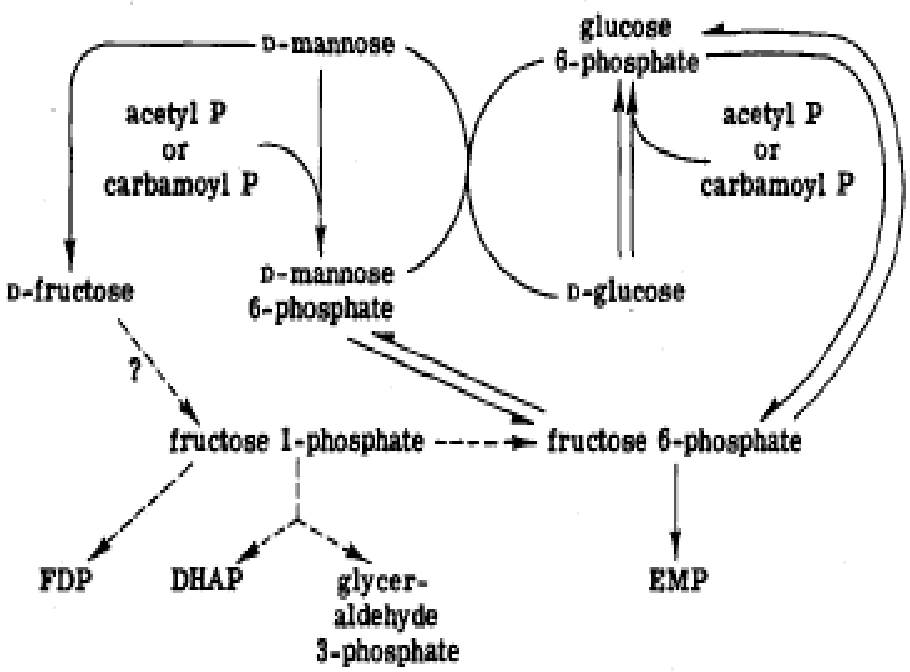
3. The third possible acetaldehyde metabolism is directed by an active alcohol dehydrogenase (alcohol:NAD oxidoreductase, EC 1.1.1.1) that converts acetaldehyde to ethanol:



Of course, there may be a number of Group N streptococci that do not follow this metabolism, as was shown with the glycine-requiring *Streptococcus cremoris* Z 8. This strain lacked the threonine aldolase.

Mannose Metabolism

The catabolism of mannose can follow two different mechanisms, a cyclic and a noncyclic mechanism for the o-isomer, whereas only the non-cyclic system of utilization appears to exist when L-mannose is the substrate.



Aerobacter aerogenes phosphorylates D-mannose with glucose 6-phosphate and phosphotransferase to mannose 6-phosphate. This phosphate transfer from glucose 6-phosphate to D-mannose also yields glucose. The same enzyme is also able to transfer the phosphate group from acetyl phosphate or carbamyl phosphate. There is no D-mannokinase present. Glucose 6-phosphate can now be regenerated by the isomerization of D-mannose 6-phosphate to fructose 6-phosphate, which is catalyzed by mannose phosphate isomerase. Fructose 6-phosphate is now converted to glucose 6-phosphate by a second isomerase.

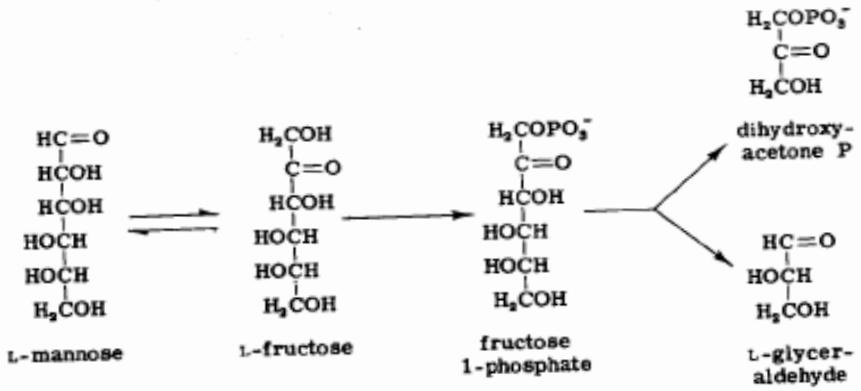
Aerobacter aerogenes PRL-R3 can also regenerate glucose 6-phosphate by phosphorylating the formed glucose with a stereospecific glucokinase. Fructose 6-phosphate is further metabolized via the EMP pathway.

The apparent epimerization of D-mannose to D-glucose may occur via a cyclic process involving D-mannose-6-phosphate isomerase, D-glucose-6-phosphate isomerase, and acylphosphate:hexose phosphotransferase.

The purification of a D-mannose isomerase from *Mycobacterium smegmalis* suggests the conversion of D-mannose to D-fructose. Whether or not D-fructose can be metabolized further via fructose 1-phosphate to fructose 6-phosphate or fructose diphosphate, as previously described with fructose metabolism, or cleaved by an aldolase as in the L-mannose utilization, is not known.

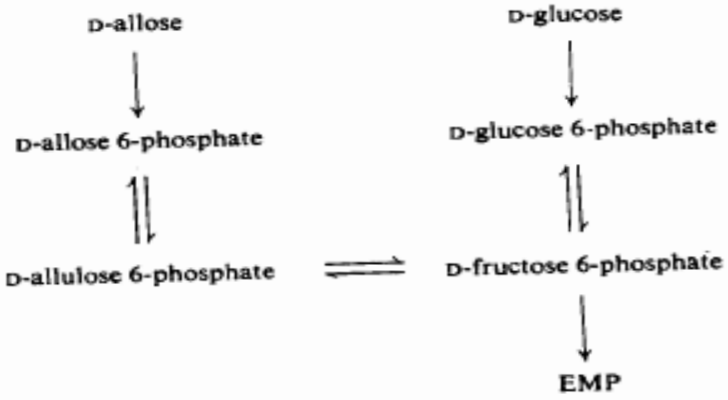
The utilization of L-mannose involves a noncyclic mechanism. L-Mannose is converted to L-fructose by a cobalt-activated isomerase.

The product of this reaction is phosphorylated at C-1 with ATP by a kinase reaction and L-fructose 1-phosphate is formed. An aldolase-type cleavage produces dihydroxyacetone phosphate and L-glyceraldehyde and thus connects this pathway with the EMP pathway.



Allose Metabolism

The catabolism of glucose and allose follow independent paths until they merge and join the EMP pathway at the fructose 6-phosphate level (144):



Because *Aerobacter aerogenes* metabolizes allose (4) under both aerobic and anaerobic conditions, most of the study of allose metabolism has been conducted with this microorganism. D-Allose is phosphorylated in the first step by a specific kinase, allose-6-kinase, to D-allose 6-phosphate. The kinase has been purified and characterized (143). The second step is per-

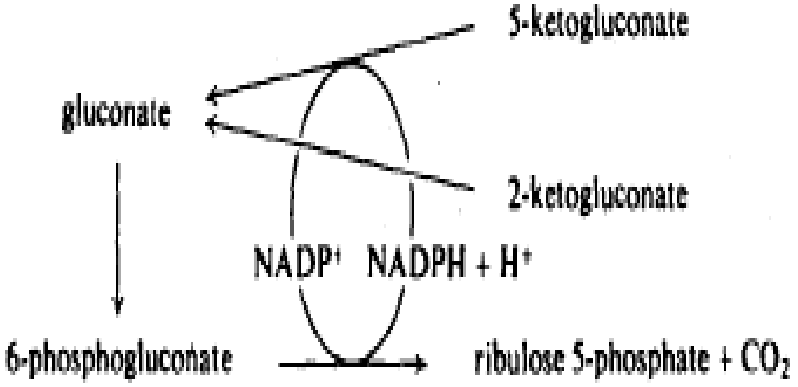
formed by an inducible isomerase, D-allose-6-phosphate ketol-isomerase and D-allulose 6-phosphate is formed. This inducible enzyme also isomerizes ribulose 5-phosphate nonspecifically to ribose 5-phosphate but does not act on other phosphorylated aldoses. The interconversion of D-allulose 6-phosphate to D-fructose 6-phosphate is thought to be catalyzed by a 3-epimerase. *Aerobacter aerogenes* is also able to metabolize glucose, for it possesses the glucosephosphate isomerase as well as a stereospecific D-glucokinase.

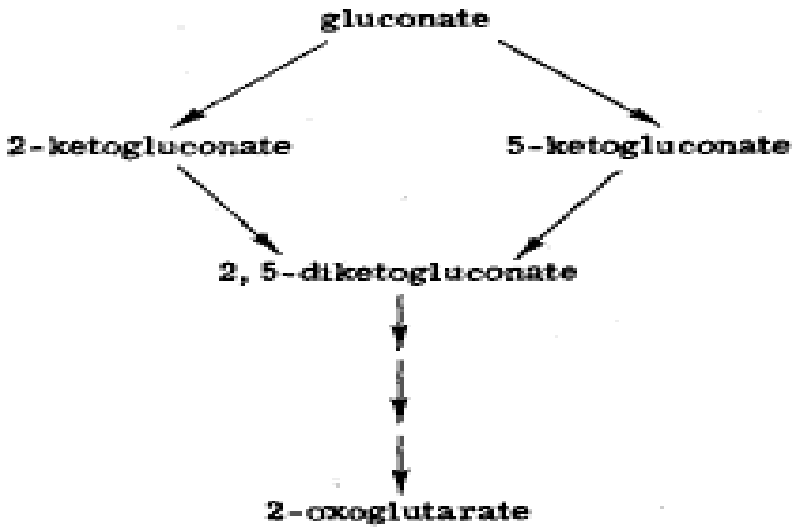
Gluconate Metabolism

A number of microorganisms are not able to phosphorylate glucose, as they lack either the hexokinase or the PEP-phosphotransferase system. Cell-free extracts of *Acetomonas suboxydans* were shown to possess two kinds of glucose dehydrogenases one DPI (2,6-dichloroindophenol) linked and a second NADP linked. It is very likely that the DPI-linked dehydrogenase is identical with the glucose oxidase which is a flavoprotein. The presence of a gluconokinase together with the 6-phosphogluconate dehydrogenase links the gluconate metabolism to the metabolism of the HMP pathway in the acetic acid bacteria.

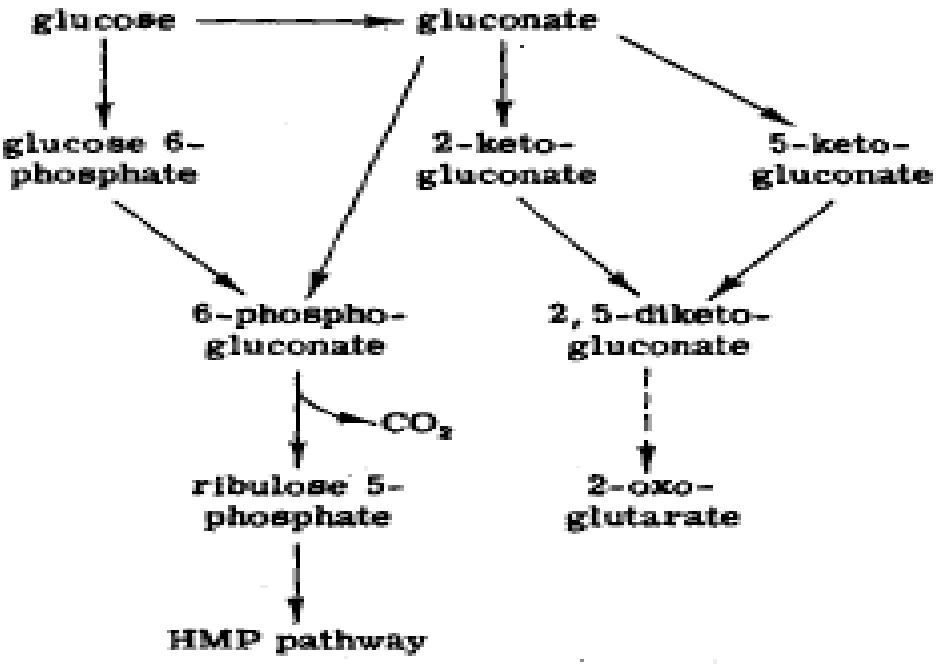
Acetic acid bacteria have a second possible path for gluconate metabolism. A 5-ketogluconate reductase metabolizes gluconate to 5-ketogluconate in *Acetomonas suboxydans*. A pH optimum of 7.5 favors the forward reaction, whereas one of 9.5 favors the reverse reaction.

Acetobacter melanogenum, however, does not possess a gluconokinase and is therefore not able to form 6-phosphogluconate. It therefore oxidizes 2-ketogluconate further, to 2,5-diketogluconate, by means of a ketogluconate dehydrogenase.

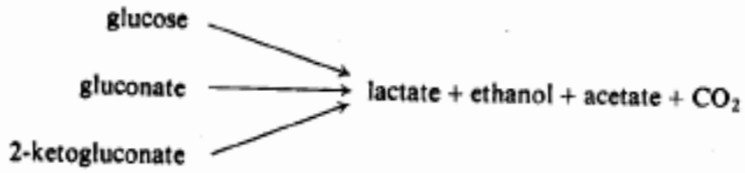




The end product of this reaction is very unstable, especially above pH 4.5, and gives rise to brown decomposition products. It therefore seems that *Acetobacter melanogenum* possesses two completely separate systems for the oxidation of glucose and hexose phosphate. Probably because of this, *A. melanogenum* is able to oxidize 2-ketogluconate further to 2,5-diketogluconate and through a series of unknown intermediates to 2-oxoglutarate. This organism has no phosphohexokinase activity. The generalized scheme of gluconate metabolism in acetic acid bacteria could be as presented in Fig.



In the lactic acid bacteria, the fermentation of hexonic acids resembles the fermentation of glucose:



Cells of *Streptococcus faecalis* grown on gluconate, however, ferment glucose (369), gluconate, and 2-ketogluconate, but glucose-grown cells were unable to attack gluconate. It was therefore assumed that a multiple pathway for gluconate fermentation must exist (131) and it was therefore postulated that after the phosphorylation of gluconate to 6-phosphogluconate had taken place, two pathways function equally for the further dissimilation of 6-phosphogluconate:

1. Oxidation of 6-phosphogluconate via the HMP and EMP pathways

$$3\ \text{6-PG} \rightarrow 3\ \text{pentose phosphate} + 3\ CO_2 + 6\ H^+\ \text{(HMP)}$$
$$3\ \text{Pentose phosphate} \rightarrow 2\ \text{F6-P} + \text{glyceraldehyde 3-P}\ \text{(HMP)}$$
$$2\ \text{F6-P} + \text{glyceraldehyde 3-P} \rightarrow 5\ \text{lactate}\ \text{(EMP)}$$

2. Oxidation of 6-phosphogluconate via the ED and EMP pathways:

$$3\ \text{6-PG} \rightarrow 3\ \text{pyruvate} + 3\ \text{glyceraldehyde 3-P}\ \text{(ED)}$$
$$3\ \text{Pyruvate} + 6\ H^+ \rightarrow 3\ \text{lactate}\ \text{(EMP)}$$
$$3\ \text{Glyceraldehyde 3-P} \rightarrow 3\ \text{lactate}\ \text{(EMP)}$$

The overall stoichiometry of these pathways would therefore be

$$\text{6-Phosphogluconate} \rightarrow 1.83\ \text{lactate} + 0.5\ CO_2$$

This has been worked out only for *Streptococcus faecalis*, which is considered a homofermentative organism. The details of the 2-ketogluconate metabolism have not been elucidated. By analogy to *Enterobacter cloacae* and *Pseudomonas fluorescens*, it would be expected that 2-ketogluconate is first phosphorylated to 2-keto-6-phosphogluconate, followed by a reduction to 6-phosphogluconate. Whether the 6-phosphogluconate is metabolized as above or heterofermentatively is still obscure. 2-Keto-6-phosphogluconate *is* also an intermediate in the gluconate metabolism of *Aerobacter cloaceae*.

The Pseudomonadaceae use the ED pathway for their gluconate utilization. *Pseudomonas fluorescens* phosphorylates not only gluconate, but also 2-

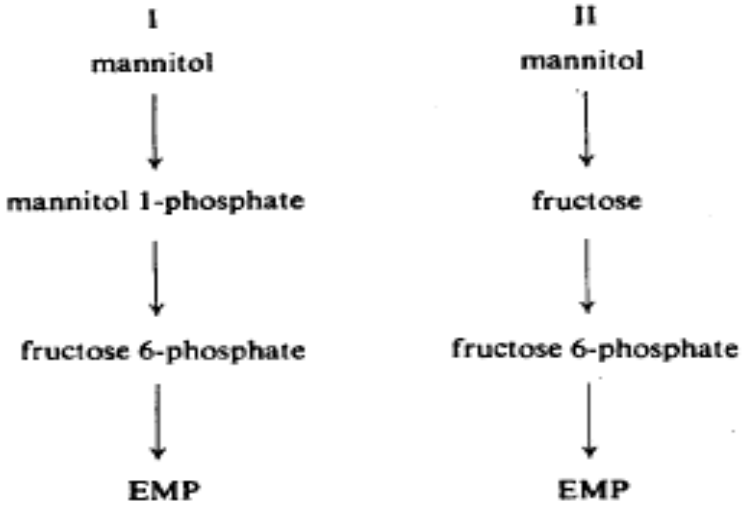
ketogluconate via 2-keto-6-phosphogluconate to 6-phos-phogluconate. The latter compound *is* then metabolized via the ED pathway.

Investigations with mutants lacking both phosphoglucose isomerase and glucose-6-phosphate dehydrogenase showed that Enterobacteriaceae, e.g., *Escherichia coli* and *Pasteurella pseudotuberculosis* appear not to possess a glucose dehydrogenase to convert glucose to gluconate. However, they metabolize gluconate via 6-phosphogluconate and the HMP or ED pathway. They also do not possess the corresponding reductases for the formation of 2-keto- or 5-ketogluconate.

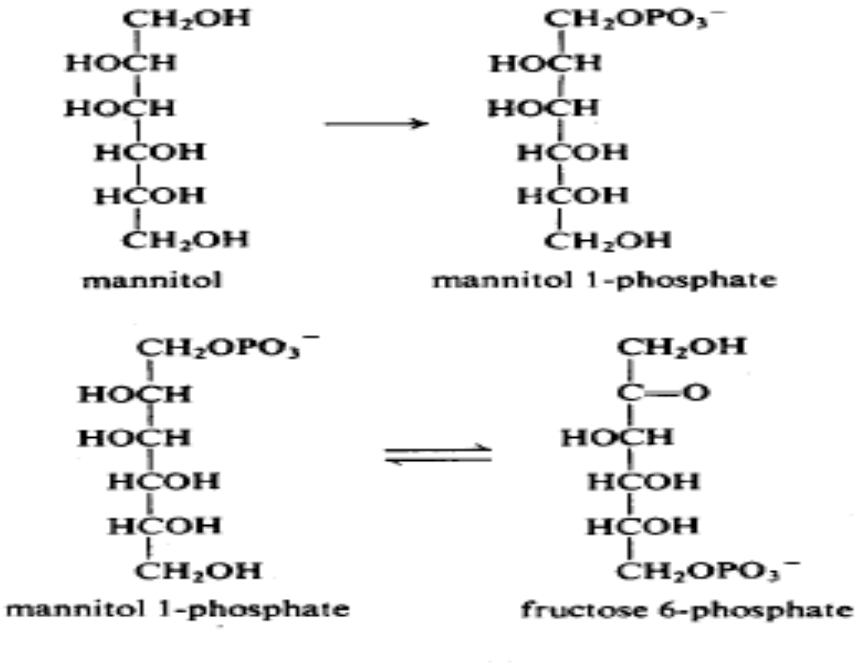
Mannitol Metabolism

Bacterial species can be divided into two groups with respect to their mode of mannitol catabolism:
(a) those which initiate it by a phos-phorylation, and
(b) those which initiate it by a dehydrogenation.

In both cases, the mannitol metabolism joins the EMP pathway at the fructose 6-phosphate level. The division into these two groups can be made on the basis of the organism's ability to form an NAD-dependent mannitol-1-phosphate dehydrogenase. With this criterion *Aerobacter aerogenes*, *Diplococcus pneumoniae*, *Escherichia coli*, *Salmonella typhimurium*, *Lactobacillus plantarum*, and *Staphylococcus aureus* would follow Scheme I, whereas *Acetobacter suboxydans* , *Azotobacter agilis*, *Cellvibrio polyoltrophicus*, and *Pseudomonas fluorescens* possess a mannitol dehydrogenase and follow Scheme II.



The first step in Scheme I, is the phosphorylation of mannitol by a mannitol kinase that is phosphoenolpyruvate dependent and converts mannitol to mannitol 1-phosphate, which can accumulate. The latter compound is now converted to the EMP pathway intermediate fructose 6-phosphate with the enzyme D-mannitol-1-phosphate dehydrogenase which is NAD linked, as catalyst.



Those microorganisms that metabolize D-mannitol to D-fructose at a nonphosphorylated level possess an NAD-linked mannitol dehydrogenase and carry out an oxidative dissimilation of mannitol.

This enzyme has been reported to be NAD linked. Whether this enzyme is identical with the cytochrome-linked mannitol dehydrogenase or with the D-mannitol: NAD+ 2-oxidoreductase is not known. The similarity with the former is striking, however, because it oxidizes D-sorbitol, as has been reported for the NAD-linked enzyme. Fructose can either be phos-phorylated by fructokinase and join the EMP pathway at the fructose 1,6-diphosphate level, as described under fructose metabolism, or be converted to glucose and gluconic acid which is characteristic for the Pseudomonadaceae.

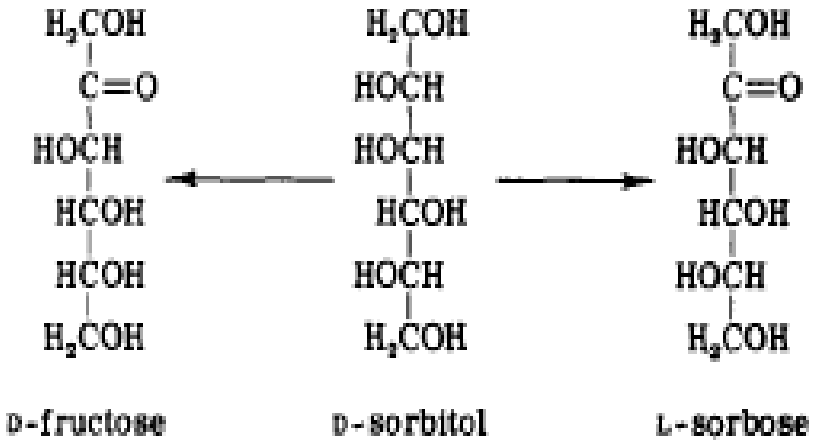
Sorbitol Metabolism

The metabolism of sorbitol is almost identical to that for mannitol because only the arrangement on C-2 differentiates between mannitol and sorbitol. The only difference occurs in the phosphorylation of sorbitol with a sorbitol kinase to sorbitol 6-phosphate. Subsequently, a NAD-linked sorbitol-6-

phosphate dehydrogenase converts sorbitol 6-phosphate to fructose 6-phosphate. Sorbitol phosphorylation is mediated by a PEP:sorbitol 6-phosphotransferase system in *Aerobacter aerogenes* PRL-R3.

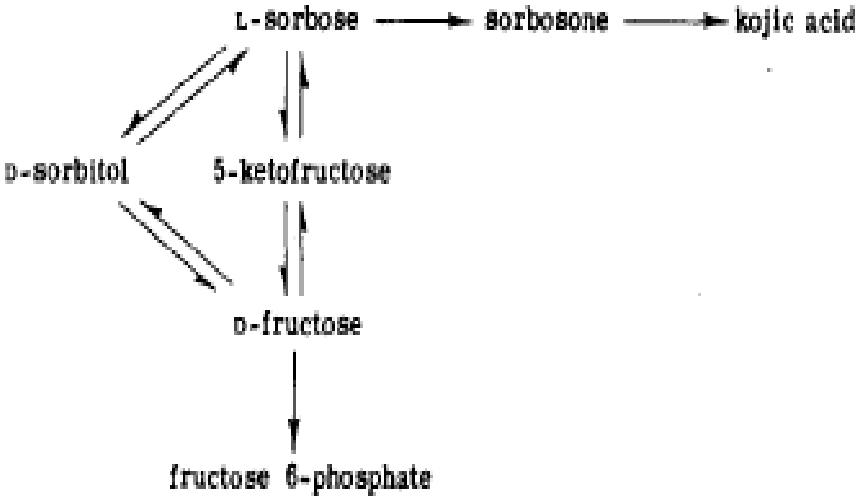
Both enzymes, mannitol-1-phosphate dehydrogenase and sorbitol-6-phosphate dehydrogenase, have been purified and found to be completely different enzymes. In *Clostridium pas-teurianum,* sorbitol-6-phosphate dehydrogenase exhibited specificity for NAD^+, NADH + H^+, sorbitol 6-phosphate, and fructose 6-phosphate. The equilibrium of the reaction lies heavily on the side of sorbitol 6-phos-phate formation.

Sorbitol itself, however, can induce the formation of mannitol-1-phosphate dehydrogenase in *Bacillus subilis* although sorbitol 6-phosphate is inactive as substrate. Acetic acid bacteria not only oxidize D-sorbitol to D-fructose in the presence of NAD^+, but also produce L-sorbose in the presence of $NADP^+$



As the NADP-linked sorbitol dehydrogenase is very unstable, the fructose formation appears more likely to occur. This would agree with the known fructose metabolism, wherein fructose is phosphorylated to fructose 6-phosphate and metabolized via the HMP pathways.

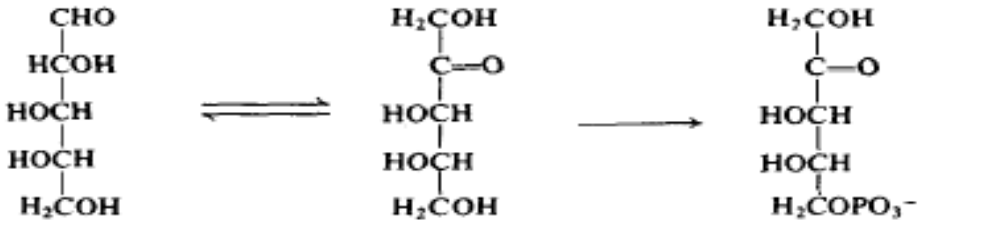
A number of acetic acid bacteria, however, appear able to oxidize L-sorbose very rapidly with the oxidation products being 5-ketofructose or kojic acid. The latter compound could also be formed from the former by *Acetobacter suboxydans* var. *nonaceticum*. This indicated that 5-ketofructose is an intermediate in the formation of kojic acid, not only from D-fructose but also from L-sorbose



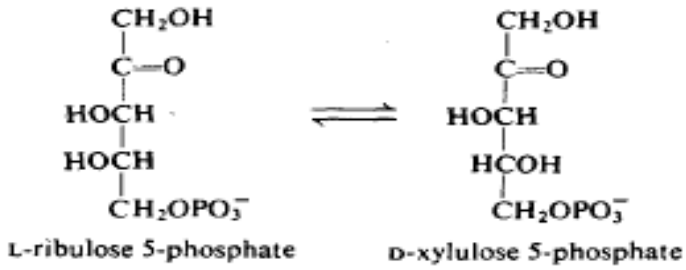
L-Arabinose Metabolism

Like D-arabinose, L-arabinose can be metabolized in three different ways.

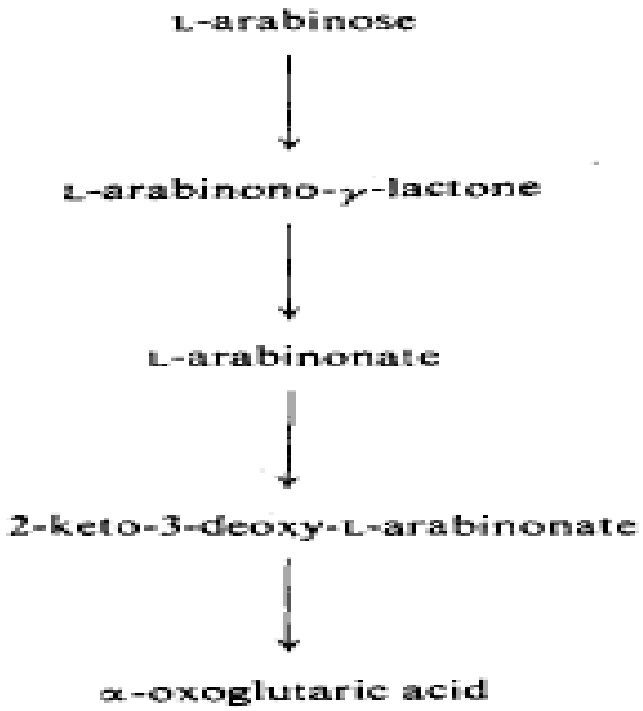
Escherichia coli, together with *Aerobacter aerogenes*, *Lactobacillus plantarum*, *Bacillus subtilis*, and *Salmonella typhimurium*, first isomerize L-arabinose to L-ribulose (362) by an isomerase action (L-arabinose ketol-isomerase, EC 5.3.1.4) (35, 306). This is then followed by a phosphorylation step. The



acting enzyme in this reaction is L-ribulokinase (235, 363) (ATP:L-ribulose 5-phosphotransferase, EC 2.7.1.16) and the product formed is L-ribulose 5-phosphate. The latter is now acted upon by a ribulosephosphate 4-epimerase (236) (L-ribulose-5-phosphate 4-epimerase, EC 5.1.3.4), which



converts L-ribulose 5-phosphate to the key intermediate D-xylulose 5-phosphate.

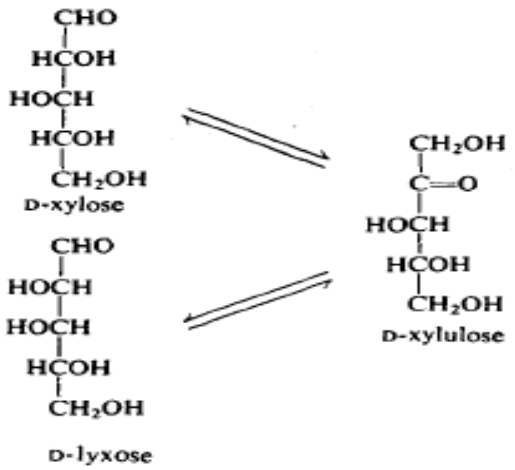


Pseudomonas saccharophila and *Pseudomonas fragi* convert L-arabinose in like manner to the D-arabinose pathway. L-Arabinose is first attacked by a dehydrogenase, forming the corresponding lactone. This reaction is NAD+ linked and produces 1 mole of reduced NAD. Arabinonolactonase hydrolyzes the lactone to L-arabinonate, which undergoes a reaction together with L-arabinonate dehydratase producing 2-keto-3-deoxy-L-arabinonate. Whereas in the ED pathway a split of the molecule occurs, this is not the case here. 2-Keto-3-deoxy-L-arabinonate with the addition of 2 H^+, forms 2-oxoglutarate. *Pseudomonas* species are therefore able to produce this dicarboxylic acid straight from L-arabinose without the formation of pyruvate.

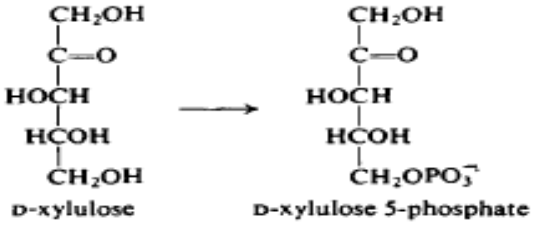
The third pathway of L-arabinose metabolism differs only in the last step. *Pseudomonas* sp. strain MSU-1 possesses a 2-keto-3-deoxy-L-arabonate aldolase, which carried out a cleavage reaction forming pyruvate and glycolaldehyde.

Xylose and Lyxose Metabolism

The conversion of D-xylose to D-xylulose is catalyzed by xylose isomerase, whereas D-lyxose is isomerized by

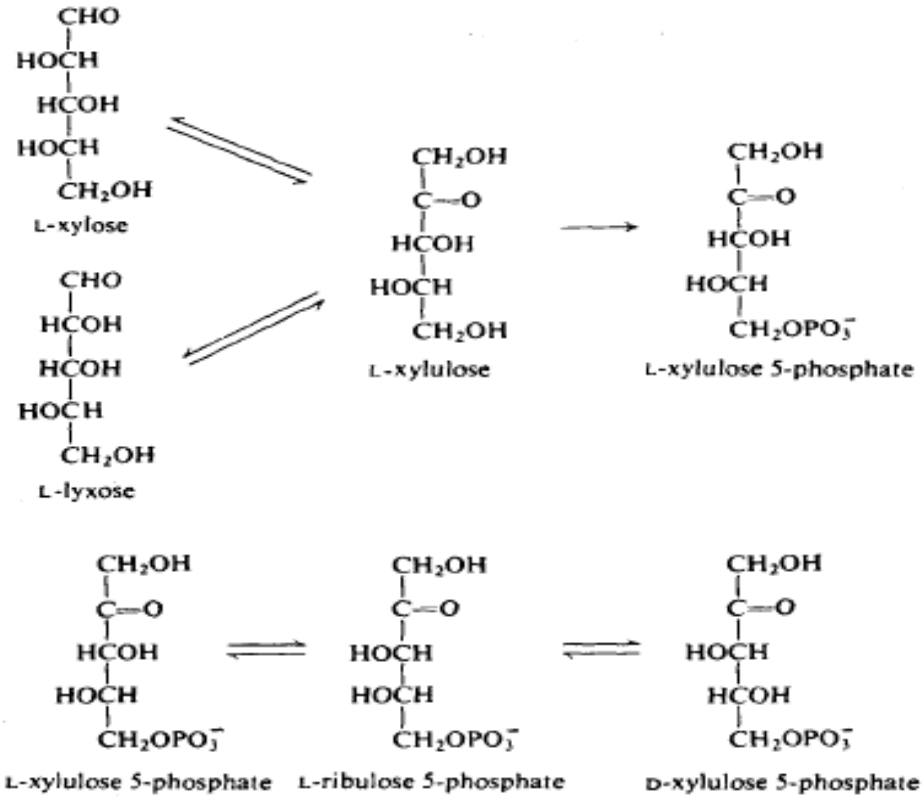


D-lyxose isomerase (D-lyxose ketol-isomerase, EC 5.3.1.15), which has been purified (8). The common product of both isomerization reactions, D-xylulose, is subsequently phosphorylated by xylulokinase (ATP:D-xylulose



5-phosphotransferase. The pathways of the L-isomers of these two pentoses have also been evaluated, but none of the isomerases is reported in the Enzyme Commission's report. It appears that the main difference between the isomerases of the D and L isomers lies in the cofactor requirements. Whereas the D-isomerases require Mg^{2+}, the L-isom-erases are reported to require Co^{2+} for their activity.

The isomerization of L-xylose and L-lyxose to L-xylulose is followed by a phosphorylation reaction, which requires ATP and Mg^{2+}, to L-xylulose 5-phosphate. The enzyme involved in this reaction is named "L-xylulo-kinase". The conversion of L-xylulose 5-phosphate to D-xylulose 5-phosphate includes two enzymatic steps, involving a 3- and a 4-epimerase. The first step in-



volves a ribulose phosphate 3-epimerase, which converts L-xylulose 5-phosphate to L-ribulose 5-phosphate. Whether or not this enzyme is identical to EC 5.1.3.1 could not be clarified. The second stage with the formation of D-xylulose 5-phosphate is the action of ribulosephosphate 4-epimerase

Polyol Metabolism of Acetic Acid Bacteria

Investigations into the polyol oxidation of *Acetomonas suboxydans* showed that this organism is able to oxidize a great number of acyclic polyols and related substances according to the rules of Bertrand. The secondary OH group involved in the oxidation must have a D configuration with respect to the primary alcohol group adjacent to the site of oxidation. *Acetomonas suboxydans* therefore contains six different polyol dehydrogenases and each of these has a unique structural specificity:

(a) $NADP^+$ - xylitol dehydrogenase

(b) NAD+ - D-mannitol dehydrogenase

(c) NAD+ - D-erythro-dehydrogenase

(d) NAD^+ -D-Xylo (D-sorbitol) dehydrogenase

(e) NADP+ -D-xylodehydrogenase

(f) $NADP^+$ -D-Lyxo (D-mannitol) dehydrogenase

Glycol Oxidation

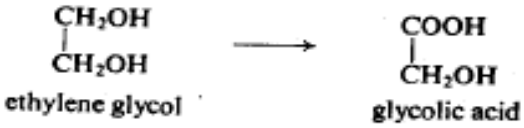
Glycerol is not only metabolized in the HMP cycle but also can function as an inducer for glycol dehydrogenases in cells originally grown on glucose or lactate medium. The formation of these enzymes seems to be especially dependent on glycerol, although a nitrogen source as well as an energy source, such as glucose, is required. The glycol oxidation of the *Acetobacter* and *Acetomonas* group appears to involve three basic enzymes:

1. A soluble NAD^+-linked primary alcohol dehydrogenase, which does not react with NADP+ but seems to require the CH—CH_2OH group because methanol is not oxidized and an OH group or a second CH_2OH group at C-2 decreases or inhibits enzymatic action. The further the second CH_2OH group is removed in position from the one to be attacked, the greater is its susceptibility to enzymatic action. The end products are most likely to be the corresponding aldehydes before strong NAD+- and NADP+-linked dehydrogenases were found to be present in *Acetomonas suboxydans.*

2. A soluble NAD^+-linked secondary alcohol dehydrogenase that attacks glycols with a secondary OH group. This enzyme seems to be less specific. The presence of an adjacent OH group improves the enzymatic activity and the end products appear to be the corresponding ketones. The presence of a third OH group, a C=O group, or a COOH group in the molecule decreases the enzymatic activity.

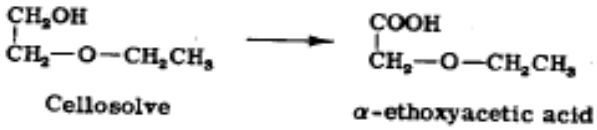
3. A participate oxidative system by which the oxidation of glycols proceeds with the uptake of oxygen. It is very likely that an electron transport system via cytochromes occurs. This oxidative system *not only oxidizes all primary and secondary alcohols* but many other compounds as well, e.g., hexoses, pentoses, polyols, and aldehydes. The oxidation of dioJs depends on the distance between *the two terminal CH_2 OH* groups. *In short-chain* diols, i.e., where these groups are situated close together, as m 1,2-ethanediol, only one of them can be oxidized. The resulting carboxyl group apparently prevents further enzymatic action. With larger molecules the negative influence of the carboxyl group decreases, and the oxidation proceeds with both types of molecules at once to the dicarboxylic acids. It appears that cytochrome 553 is tightly bonded to the enzyme aggregate. There is no evidence for the participation of NAD, NADP, flavins, ubi-quinones, or free heavy metals in this system.

Acetobacter xylinum, *A. aceti*, *A. acetosus*, *A. kuetzingianus*, *A. pasteurianus*, *A. acetigenus*, *A. ascendens*, *Acetomonas suboxydans* (*oxydans*), and *Acetomonas industrium* are able to oxidize ethylene glycol to glycolic acid, with glycolic acetaldehyde as a possible intermediate:

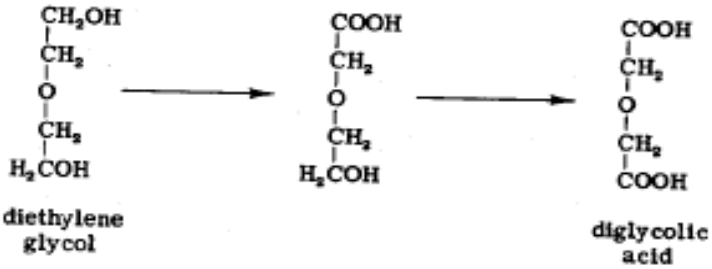


The mechanism by which this C-2 substrate is converted into cell material, however, is still unknown. Ethylene glycol monomethyl ether (Methyl Cellosolve) is quickly oxidized by resting cells of *Acetomonas suboxydans*

with the uptake of 1 mole of O_2 per mole substrate and with the possible formation of α-methoxyacetic acid. The monoethyl ether of ethylene glycol (Cellosolve) is oxidized very slowly, and therefore this oxidation never comes to completion with submerged cultures of *Acetomonas suboxydans*

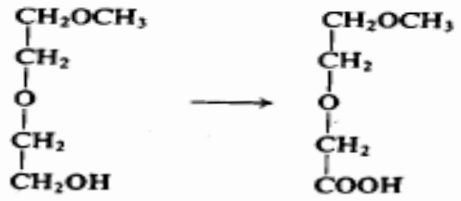


(184, 314). The α-ethoxyacetic acid is formed. An incomplete oxidation occurs also with diethylene glycol in aerated, submerged cultures of *Acetomonas suboxydans* with the formation of α-hydroxyethoxyacetic



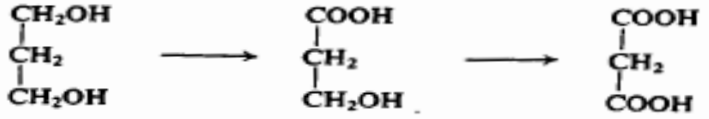
Acid and corrospondsing dicarboxylic acid.

The monomethyl ether of diethylene glycol can be oxidized to yield ethoxyacetic acid.



monomethyl ether of diethylene glycol — 2-(2-methoxy)-ethoxy acid

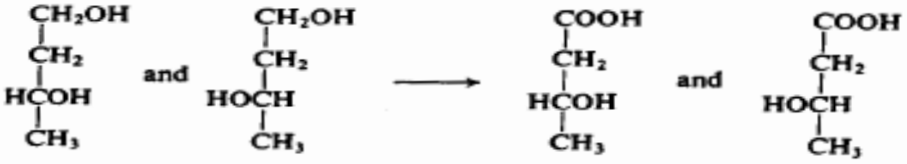
is only 36%. Triethylene glycol is oxidized very rapidly by resting cells of *Acetomonas suboxydans*, with an uptake of 1 mole of O_2 per mole substrate, probably to a carboxyl group on one end of the molecule. This is followed by a slower oxidation to another carboxyl group at the other end (209). *Acetomonas melanogenum* grows rapidly on trimethylene glycol (1,3-propanediol) and oxidizes this compound to malonic acid with hydra-



1,3-propanol — malonic acid

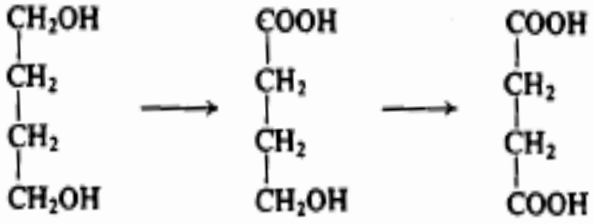
crylic acid (β-hydroxypropionic acid) as an intermediate. *Acetomonas suboxydans* is only able to grow on and utilize this compound very slowly.

DL-1,3-Butanediol seems to be a good substrate for all acetic acid bacteria because all strains studied to date are able to oxidize it to DL-β-hydroxybutyric acid.



1,3-butanediol — β-hydroxybutyric acid

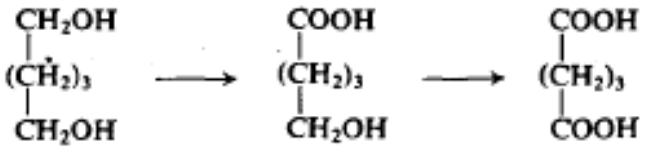
Acetomonas strains are able to oxidize 1,4-butanediol (tetramethylene glycol) to succinic acid with γ-hydroxybutyric acid as intermediate.



1,4-butanediol — γ-hydroxybutyric acid — succinic acid

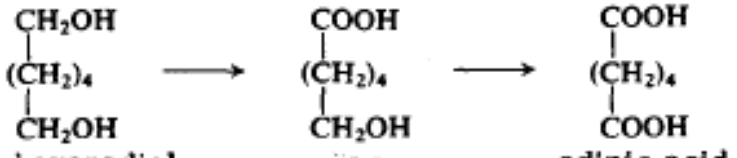
Strains of the *Acetobacter mesoxydans*, *A. oxydans*, and *A. peroxydans* group can oxidize succinic acid even further through the TCA cycle.

Glutaric acid is produced in two steps from 1,5-pentanediol by *Acetomonas*

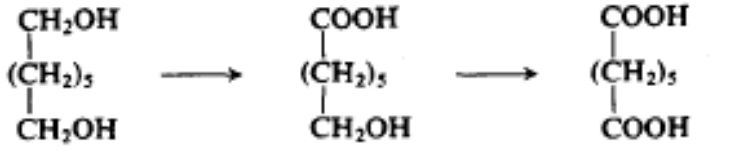


1, 5-pentanediol glutaric acid

suboxydans, forming β-hydroxyvaleric acid as intermediate. The same oxidation applies for 1,6-hexanediol, which oxidizes to adipic acid via a



1, 6-hexanediol adipic acid

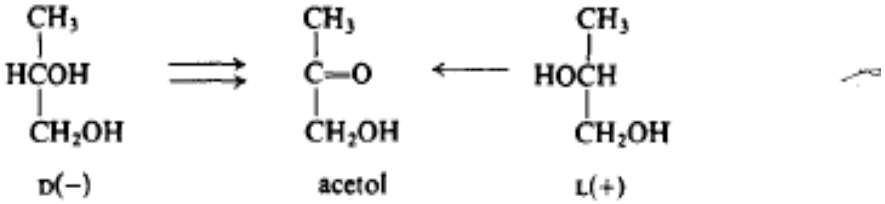


1, 7-heptanediol pimelic acid

hydroxycaproic acid, and for 1,7-heptanediol, which forms pimelic acid with 7-hydroxyheptylic acid as intermediate.

Oxidation of Secondary Alcohol Groups

Experiments have shown that the mode of attack on secondary alcohol groups depends on the distance between both groups in the molecule. When groups are adjacent, as in 1,2-propanediol, only secondary alcohol groups are oxidized:



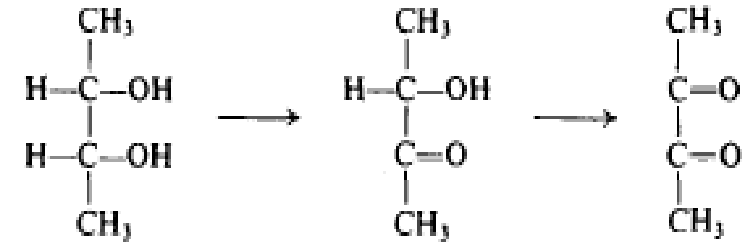
D(−) acetol L(+)

When both groups are separated by a CH_2 group, as in 1,3-butanediol, the opposite happens. The primary alcohol group is oxidized but the secondary CHOH group is not attacked at all.

2,3-Butanediol Metabolism

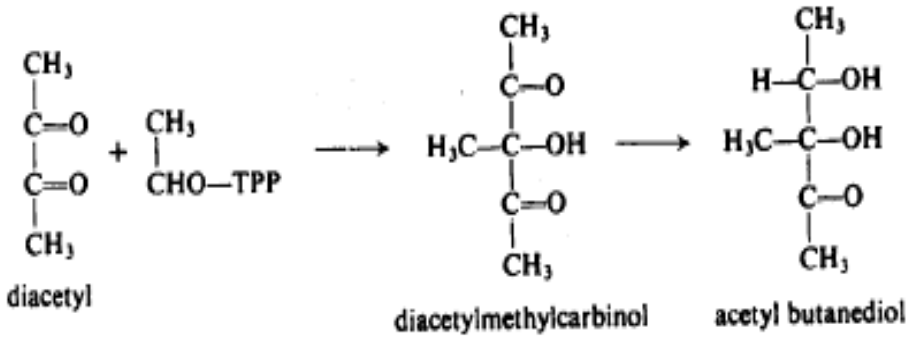
As will be discussed under fermentation, 2,3-butanediol is a major end product of carbohydrate metabolism in *Aerobacter* and *Bacillus* and can be broken down aerobically by pseudomonads. Almost all other oxidation reactions involving 2,3-butanediol can also take place anaerobically (190).

Butanediol dehydrogenase (2,3-butanediol:NAD oxidoreductase, EC 1.1.1.4) oxidizes 2,3-butanediol to acetoin. This is followed by a second oxidation step, catalyzed by acetoin dehydrogenase (acetoin:NAD oxidoreductase, EC 1.1.1.5).



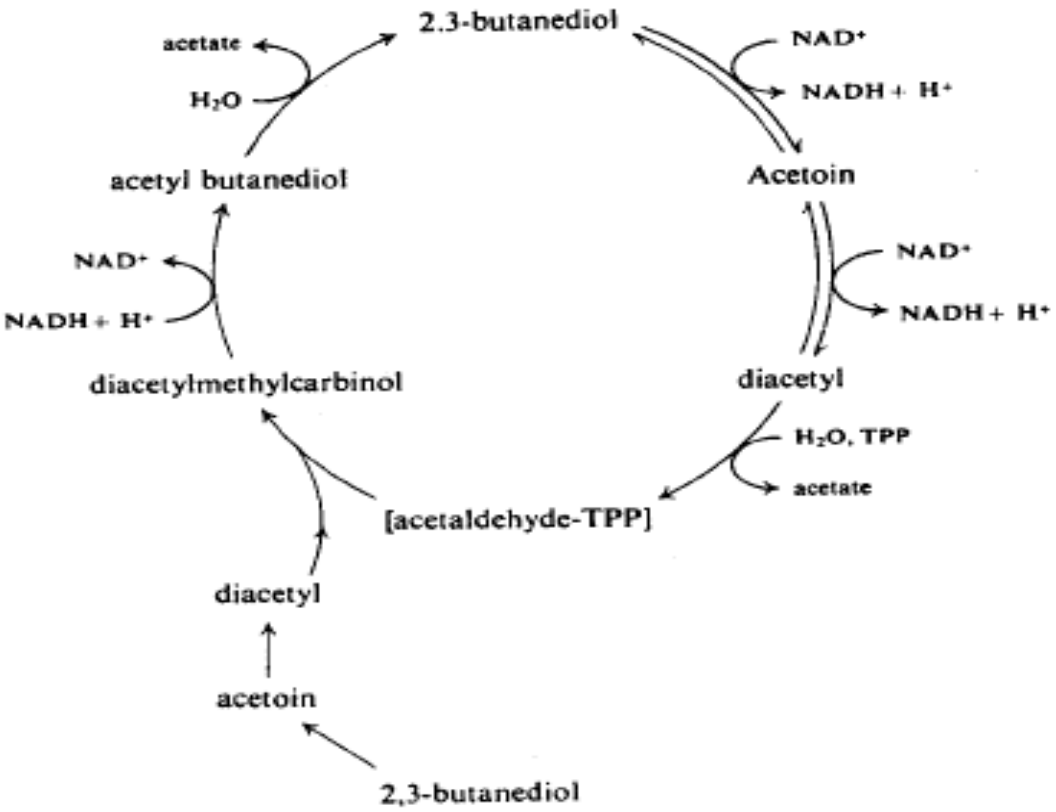
The formation of acetate and acetaldehyde–TPP complex and the combination of a second molecule of diacetyl with this complex are analogous to the synthesis of α-acetolactate from pyruvate, but the enzymes are specific and are not interchangeable with the pyruvate enzyme system of *Aerobacter* (Fig. 5.16).

Diacetyl, together with acetaldehyde–TPP, forms diacetyl methylcarbinol and a diacetylmethylcarbinol reductase that is probably very similar to butanediol dehydrogenase (EC 1.1.1.4) because it is NAD^+-linked (191), reduces diacetyl methylcarbinol to acetylbutanediol. Hydrolysis now occurs and, in a step similar to that of acetaldehyde–TPP formation, a further molecule of acetate is formed, together with butanediol. In this cyclic process, two molecules of acetate are formed from two mole-



cules of 2,3-butanediol. It seems probable that the same cycle is operative in *Pseudomonas fluorescens* (190) and *Pseudomonas* sp. (186). The acetate produced is used for synthesizing cell material in processes involving the TCA and glyoxylate cycles.

The mechanism of oxidation of 2,3-butanediol and acetoin in acetic acid bacteria is not as yet clear. According to Bertrand's rule, only the meso form should be oxidized. However, experiments revealed that both



Growth with C1 Compounds

In connection with the energy metabolism of aerobes it was mentioned that a few groups of bacteria cannot employ the tricarboxylic acid cycle for the production of reducing power. One of these groups comprises the microorganisms growing on C1 compounds. Two subgroups can be envisaged:

A. Obligate methylotrophs, which grow only at the expense of compounds containing no carbon-carbon bonds (methane, methanol, etc.). This group includes all methanotrophs.

B. Facultative methylotrophs, which grow on a variety of carbon sources including Q compounds. These organisms utilize methanol and methylamine but not methane.

A. Obligate methylotrophs

The first methylotroph isolated was *Bacillus methanicus*. Fiftyyears later it was reisolated and named *Pseudomonas methanica.* During the last 10 years a large number of methylotrophic bacteria became known through the work of Whittenbury and Wilkinson; they are distinguished as *Methylosinus, Melhylocystis, Methylomonas, Methylobacter,* and *Methylococcus.* Ultrathin sections of cells of these species revealed that they contain extensive membrane structures. It is also noteworthy that all these species form resting stages, in most cases cysts, but some species form thermostable exospores.

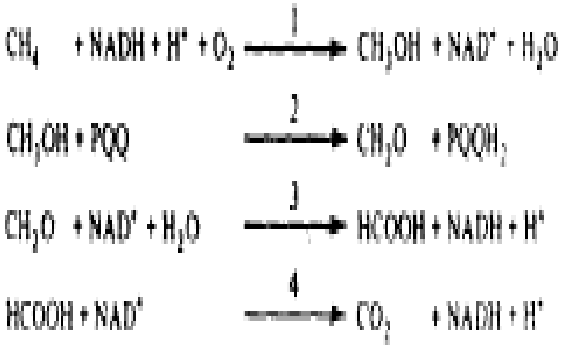
Clearly, during growth on methane, reducing power for the respiratory chain can be produce'd only by oxidation of methane to CO_2. There is no acetyl-CoA available to be oxidized via the tricarboxylic acid cycle. Oxidation of methane proceeds as shown in Fig. 6.18. Methane is first oxidized by a monooxygenase to methanol. NADH functions as cosubstrate in this reaction. Methanol dehydrogenase is not coupled to the reduction of NAD"[1]" but to electron transfer to a novel coenzyme—methoxatin (PQQ). It is evident from its chemical structure that this compound is an *ortho*-quinone. By reduction the corresponding hydroquinone is formed. The redox potential of PQQ is E'_0 = +120 mV, so it is more suitable as H-acceptor in a number of dehydrogenation reactions than NAD^+. That is the reason why it also functions as coenzyme in the glucose dehydrogenase reaction and in other systems.

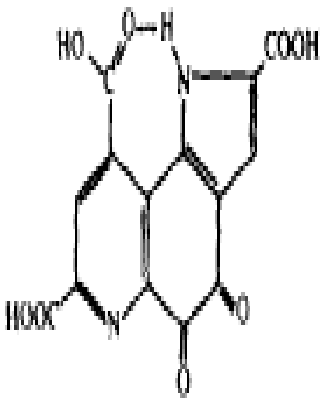
The oxidation of formaldehyde to CO_2 proceeds in two NAD^+ -linked steps. One of the NADH formed has to be invested by the organism into the monooxygenase reaction. The second NADH is fed into the respiratory chain,

as is $PQQH_2$, the latter, however, at the level of cytochrome *c*. It is noteworthy that methane monooxygenase does not exhibit an absolute substrate specificity. For instance, it oxidizes ammonia to hydroxylamine, but is definitely different from the true ammonia monooxygenase.

The anabolic metabolism of the methylotrophs diverges from the energy metabolism at the level of formaldehyde. Three formaldehyde fixation cycles have been elucidated, largely due to work done in the laboratories of Quayle, Hersh, and Harder.

1. The serine-isocitrate lyase pathway. The formation of acetyl-CoA from formaldehyde and CO_2 in the serine pathway is shown in Fig. 6.19. The acceptor for formaldehyde is glycine, and the serine formed in the hydroxymethylase reaction is converted to the corresponding α-oxo acid.





PQQ

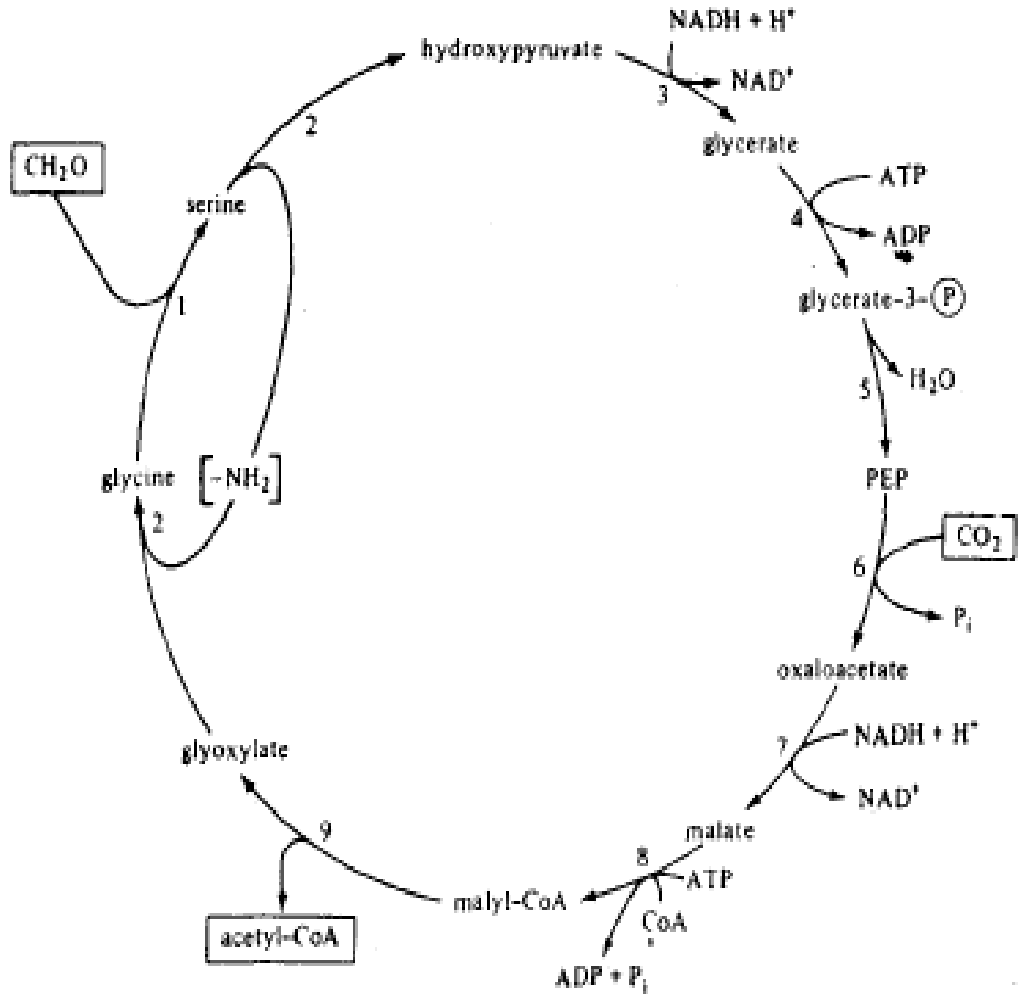
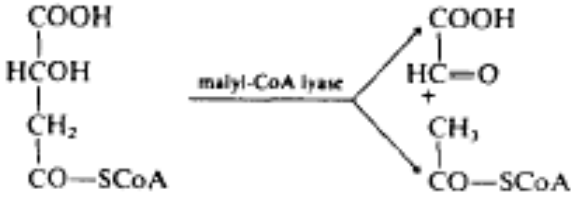


Figure 6.19. Formation of acetyl-CoA from formaldehyde and CO_2 in the serine pathway. 1, Serine hydroxymethylase; 2, a transaminase that converts serine into hydroxypyruvate and glyoxylate into glycine; 3, hydroxypyruvate reductase; 4, glycerate kinase; 5, phosphoglycerate mutase and enolase; 6, PEP carboxylase; 7, malate dehydrogenase; 8, malyl-CoA synthetase; 9, malyl-CoA lyase.

via a transaminase reaction. Hydroxypyruvate is then further converted to PEP by the action of several enzymes. Oxaloacetate is formed through the PEP carboxylase reaction. The key enzyme for the generation of glyoxylate—malyl-CoA lyase—came under study only recently, despite the fact that this cleavage reaction was discovered 15 years ago in *Rhodopseudomonas sphaeroides*. It converts malyl-CoA into acetyl-CoA and glyoxylate. Before this reaction can proceed, L-malate has to be activated. This is done by the action of a specific synthetase, malyl-CoA synthetase. The β-carboxyl group of L-malate is converted into a CoA-thioester group. This conversion is associated with the formation of ADP and P_i from ATP. Besides malyl-CoA synthetase and lyase two other enzymes can be considered as key enzymes of this cycle, serine-glyoxylate transaminase and hydroxypyruvate reductase.



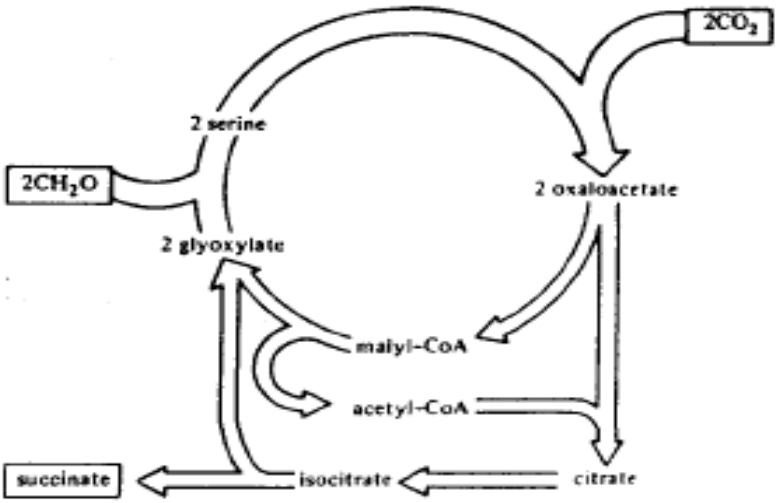
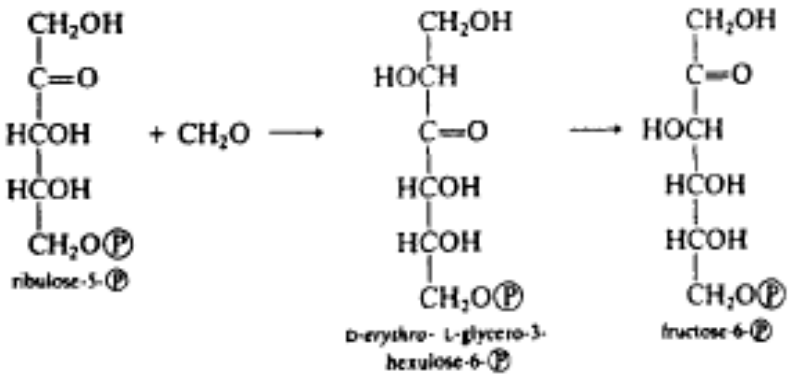


Figure 6.20. Net formation of succinate from formaldehyde and CO_2 by the serine-isocitrate lyase pathway.

The result of the serine pathway is the formation of acetyl-CoA from CH_2O and CO_2. The cycle is insufficient to supply the cell with PEP or oxaloacetate for biosynthetic purposes. This, however, is achieved if it is combined with the citrate synthase, *cis*-aconitase, and isocitrate lyase reactions (Fig. 6.20). In essence, this accomplishes the net formation of succinate from $2CO_2$ and 2 formaldehyde. The serine-isocitrate lyase pathway is present in *Methylosinus* and *Methylocystis* and a number of facultative C_1 utilizers.

2. The ribulose-monophosphate cycle. The key reactions of this cycle are the condensation of ribulose-5-phosphate and formaldehyde by hexulose-6-phosphate synthase and the isomerization of the product to fructose-6-phosphate.



The acceptor—ribulose-5-phosphate—is regenerated through a series of reactions depicted in Fig. 6.21. Three molecules of formaldehyde finally end up in one molecule of dihydroxyacetonephosphate, which can be utilized for biosynthetic purposes. The ribulose-monophosphate cycle occurs in *Methylococcus* and *Methylomonas* species.

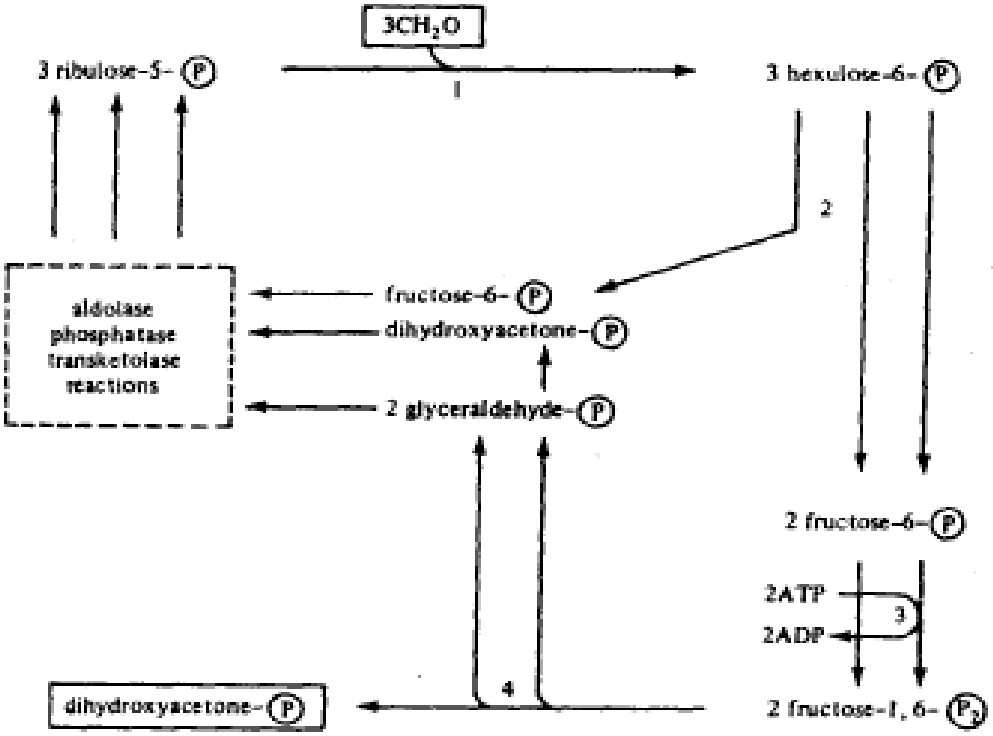


Figure 6.21. The ribulose-monophosphate cycle. 1, Hexulose-6-phosphate synthase; 2, hexulose-6-phosphate isomerase; 3, phosphofructokinase; 4, fructose-1,6-bisphosphate aldolase.

In detail, the cycle operates as follows: Two of the three molecules of fructose-6-phosphate are phosphorylated and the resulting fructose-1,6-bisphosphate is cleaved by aldolase into dihydroxyacetonephosphate and

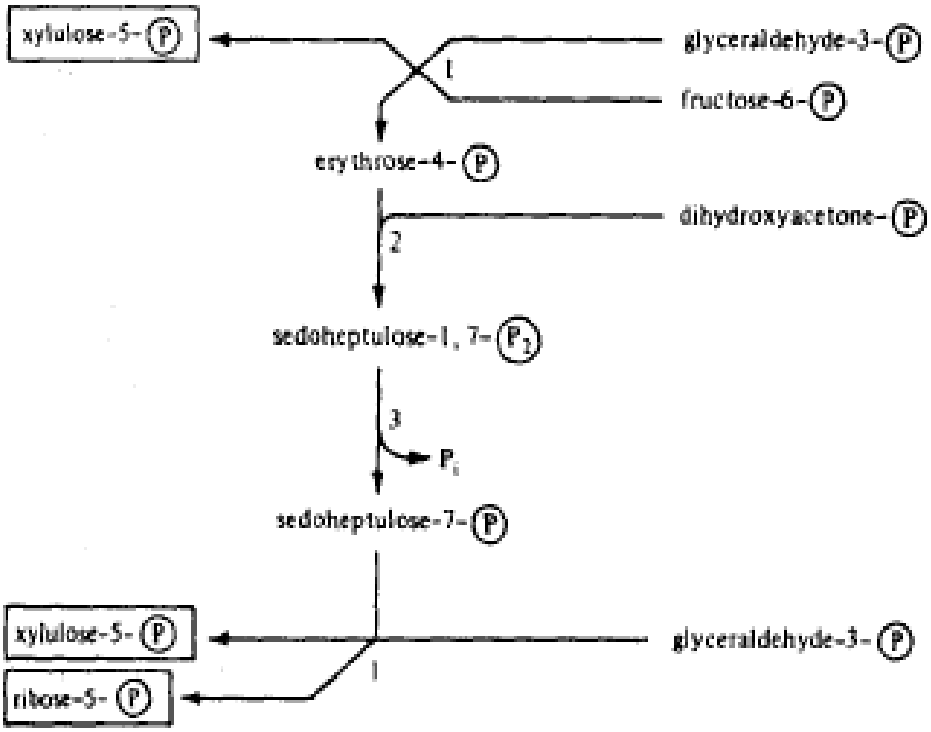
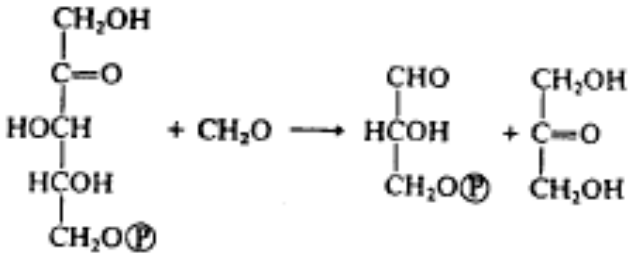


Figure 6.22. The conversion of fructose-6-phosphate, glyceraldehyde-3-phosphate, and dihydroxyacetonephosphate into pentose phosphate. 1, Transketolase; 2, aldolase; 3, phosphatase.

glyceraldehyde-3-phosphate. Two glyceraldehyde phosphate, one dihydroxyacetonephosphate, and one fructose-6-phosphate undergo a series of reactions that resemble the reactions of the pentose phosphate cycle (see Fig. 3.14). The difference is that the reaction sequence here is unidirectional; it proceeds only in the direction: hexosephosphates → pentosephosphates, but not vice versa. The reason for this is that sedoheptulose-7-phosphate is not formed from erythrose-4-phosphate in a transaldolase reaction but in a true aldolase reaction with dihydroxyacetonephosphate as the second substrate (Fig. 6.22). Sedoheptulose-1, 7-bisphosphate is converted to the 7-phosphate by a phosphatase. This reaction is irreversible and determines the direction of the entire sequence.

3. Xylulose-monophosphate cycle. In yeast, but not in bacteria, a third formaldehyde fixation cycle has been found to occur (Fig. 6.23). Its key reaction is a specific transketolase reaction by which the first two carbon atoms of xylulose-5-phosphate are transferred to formaldehyde:



Dihydroxyacetone is formed which is phosphorylated to dihydroxyacetone phosphate by a specific kinase that can be regarded as the second key

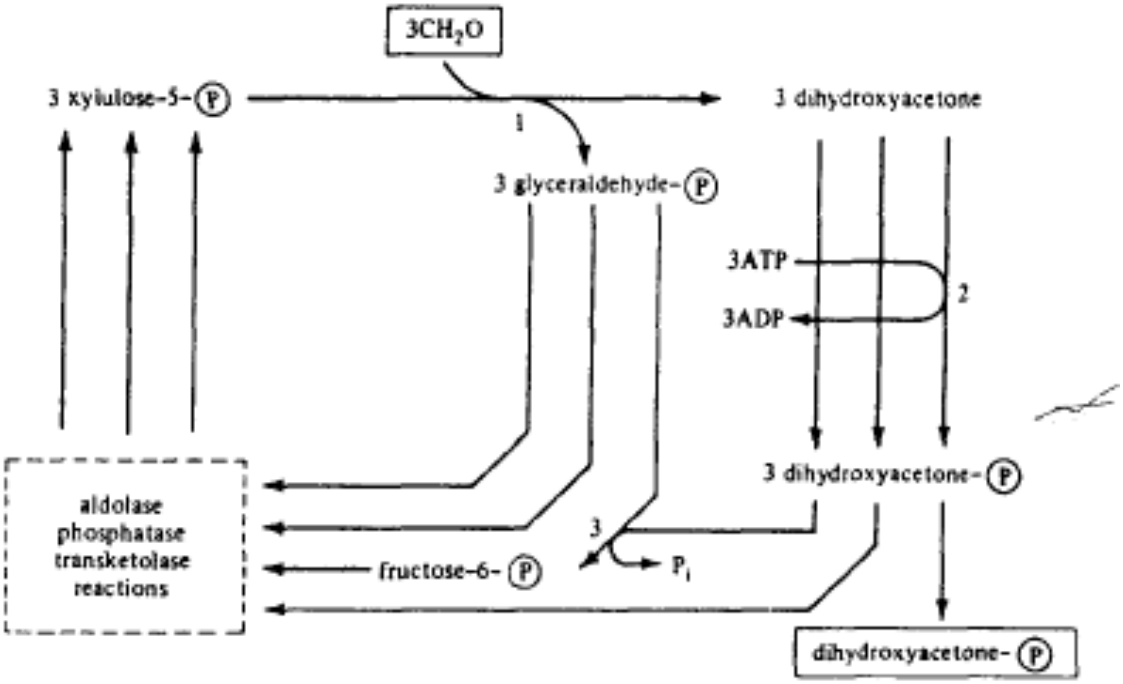


Figure 6.23. The xylulose-monophosphate cycle. 1, Dihydroxyacetone synthase; 2, dihydroxyacetone kinase (triakinase); 3, fructose-1,6-bisphosphate aldolase + fructose-1,6-bisphosphatase.

enzyme of this cycle. All further reactions are very much similar to the ones of the ribulose-monophosphate cycle.

It should be mentioned that formaldehyde is a very suitable C, precursor for the synthesis of cellular material. Its redox grade is the same as the one of sugar. Its conversion to glyceraldehyde-3-phosphate requires 2-4 ATP (depending on the pathway). For comparison, the fixation and reduction of $3CO_2$ to glyceraldehyde-3-phosphate requires 9ATP.

A few additional remarks with respect to the methylotrophic yeasts are necessary. The ability to grow on methanol is not very widespread among yeasts. Only some *Candida, Hansenula,* and *Torulopsis* species are able to do so. They employ the xylulose-monophosphate cycle for formaldehyde fixation. It is also noteworthy that these yeasts do not contain a PQQ-dependent methanol dehydrogenase. Instead, a methanol oxidase is acting on methanol:

$$CH_3OH + O_2 \longrightarrow CH_2O + H_2O_2$$

A catalase takes care of the H_2O_2 formed in this reaction. Both enzymes, methanol oxidase and catalase, reside in special organelles, the peroxi-somes. Formaldehyde produced diffuses out of these peroxisomes and is oxidized further in the cytoplasm.

B. Facultative methylotrophs

A number of microprganisms besides the obligate methylotrophs are able to grow with C_t compounds and more complex organic compounds. Among these are yeasts, *Hyphomicrvbium* species, pseudomonads such as *P. oxalaticus* and *P.* AMI, and *Protaminobacter* species. These organisms utilize methanol, formate, trimethylamine, or methylamine, but are unable to grow with methane. Consequently, growth on methane is the domain of the obligate methylotrophs.

Methylamines are important substrates in nature. Several enzyme systems have been characterized '.hat oxidize these compounds. A flavoprotein acting on trimethylamir.e catalyses the following reaction:

$$(CH_3)_3N + H_2O + FAD \longrightarrow (CH_3)_2NH + CH_2O + FADH_2$$

The reduced flavoprotein is reoxid.ized by reaction with components of the respiratory chain. Further oxidation cf dimethylamine and monomethyla-mine also yields formaldehyde as the principal product.

When growing with methanol, prokaryotic facultative methylotrophs employ either the serine-isocitratc lyase pathway or the ribulose

monophosphate cycle. Formate is assimilated by *P. oxalaticus* via CO_2 and the ribulose-l,5-bisphosphate cycle. Yeasts use, as outlined, the xylulose-monophosphate pathway.

C. Methanol as substrate for the production of single-cell protein

The use of methanol as substrate for single-cell protein production has several advantages; it is cheap and available in high purity; it is water soluble and allows faster growth of methylotrophs than methane; O_2- and NADH-consuming monooxygenase reactions are not involved in substrate oxidation. The commercial processes for the production of single-cell protein are based on the use of *Methylomonas* or *Methylophilus* species. Yeasts play only a minor role.