Microbial cell metabolism

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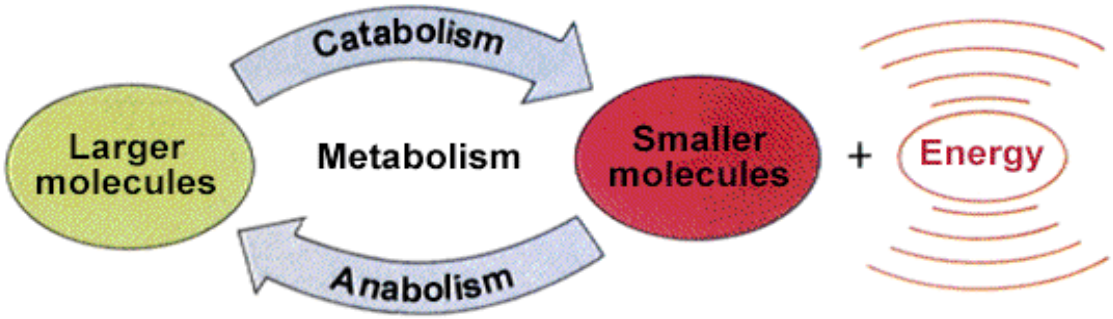
- Metabolic reactions are the chemical reactions that occur in the microbial cell,
- It may be constructive (Anabolic) or destructive processes

1. **Anabolism: Synthesis of more complex compounds and use of energy**
2. **Catabolism: Break down a substrate and capture energy.**


Catabolism
Larger molecules
Metabolism
Smaller molecules
+
Energy
Anabolism

Microbial metabolism can be divided into four general categories:

- 1- pathway for the interconversion of focal metabolites .
- 2- Assimilatory pathways for the formation of focal metabolites.

- 3- Biosynthesis sequences for the conversion of focal metabolites to end products.
- 4- Pathways for metabolic energy yield for growth and maintenance.

- A macromolecule is determined in one of the two ways :
- in nucleic acids and proteins , its template directed :
- DNA serves as the template for its own synthesis and for the synthesis of the various types of RNA.

- In carbohydrates and lipids, the arrangement of building blocks is determined entirely by enzyme specificities.

- Microbial growth requires the polymerization of biochemical building blocks into proteins , nucleic acids , polysaccharides . lipids etc.
- These building blocks must be supplied in the culture medium or synthesized by the growing cells.

- Metabolic ways require other factors in addition to building blocks like vitamins and enzymes .
- Building blocks and coenzymes can be traced to relatively few precursors called Focal metabolites.
- Such focal metabolites should be present in the cell for the incitation of biochemical reactions .

- focal metabolites give rise to intermediates and finally to end products .
- the most important Focal metabolites are:
- 1- Glucose 6 phosphate(G6P).

  It is the yield of glucose activation by kinase enzyme with the aid of Mg ions as catalyst.

- This compound has many intermediated compounds like Hexose –p,
- Pentose –p, triose –p and pyrovate . its end products are either proteins , fatty acids or nucleic acids .

metabolites.

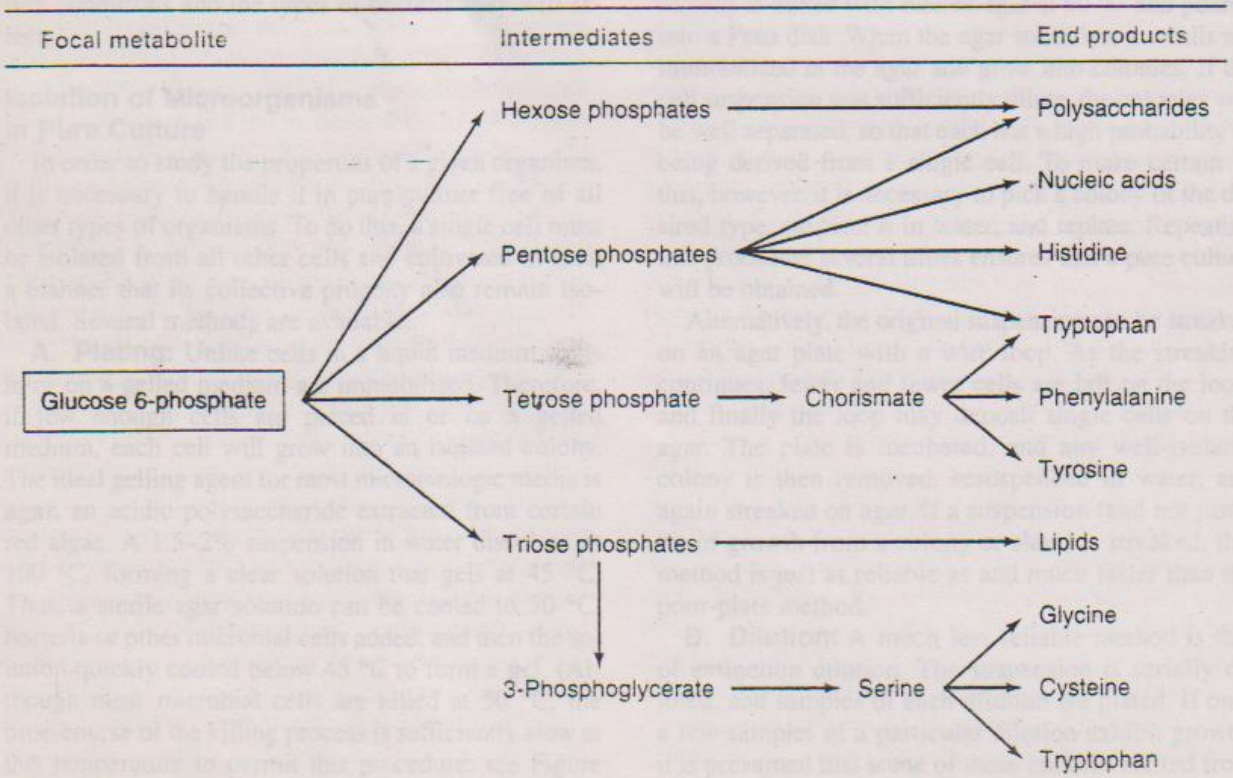


Figure 6–1. Biosynthetic end products formed from glucose 6-phosphate. Carbohydrate phosphate esters of varying chain length serve as intermediates in the biosynthetic pathways.

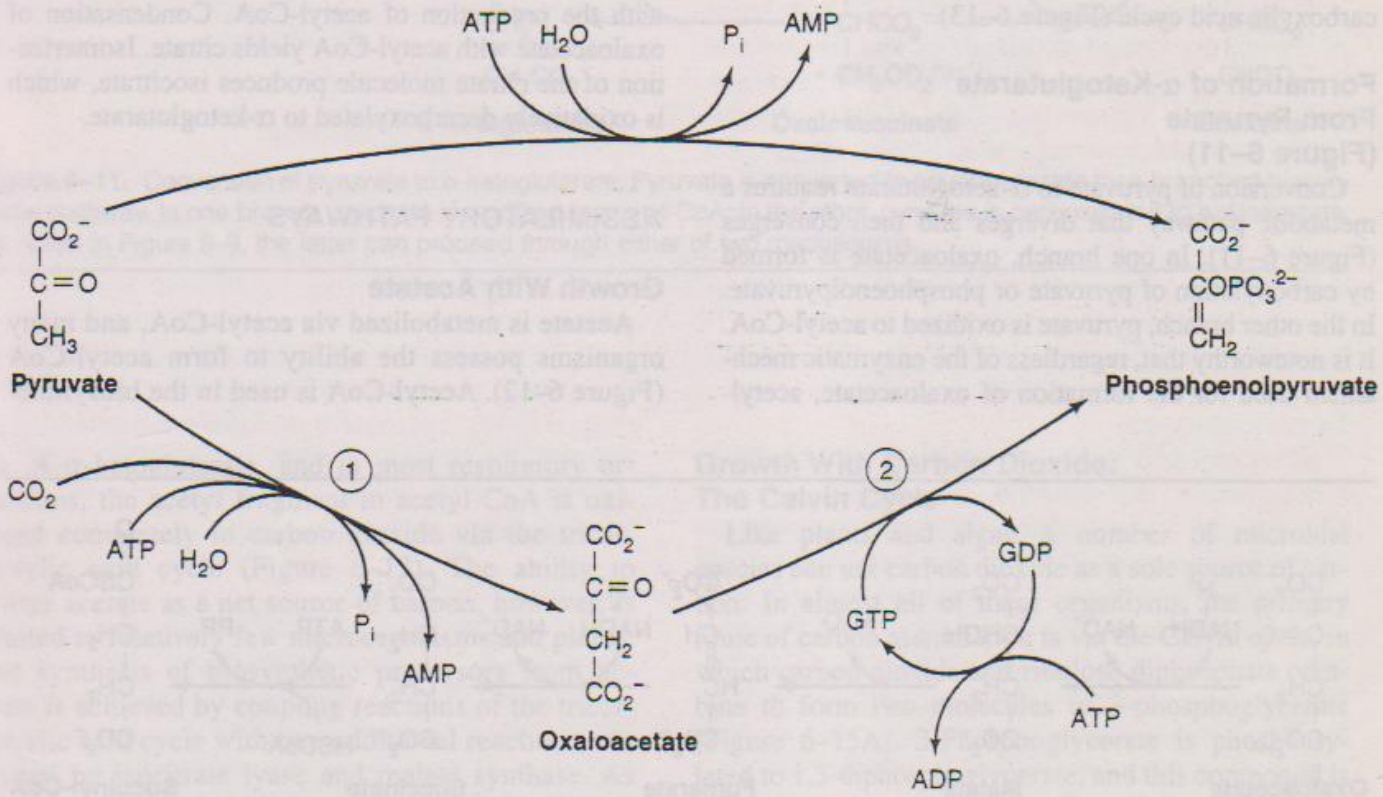
- 2- Phospho Enol Pyrovate also resembles an important focal metabolite in microbes .

Formation and utilization of phosphoenol pyrovate.

- **Formation of phosphoenolpyrovate from pyrovate requires amount of metabolic energy, and two ATP bonds as shown below:**

- 1-some microbes carry out the reaction in a single step in which the phosphorylation of pyrovate is enzymatically coupled to the hydrolysis of phosphate bond .

- other organisms invest pyrophosphate bonds in each of two consecutive metabolic steps:
- A- the ATP dependent carboxylation of pyrophosphate to oxaloacetate.
- B- The GTP- dependent decarboxylation of oxaloacetate to Phosphoenolpyrovate


ATP
H_2O
P_i
AMP
CO_2^-
C=O
CH_3
Pyruvate
CO_2^-
$COPO_3^{2-}$
CH_2
Phosphoenolpyruvate
CO_2
ATP
H_2O
1
P_i
AMP
CO_2^-
C=O
CH_2
CO_2^-
Oxaloacetate
2
GDP
GTP
ATP
ADP

Biosynthetic end products formed from Phosphoenol pyrovate.

- **Phosphoenolpyrovate intermediate compounds are triose phosphate while the end products are similar to that of Glucose 6 phosphate.**

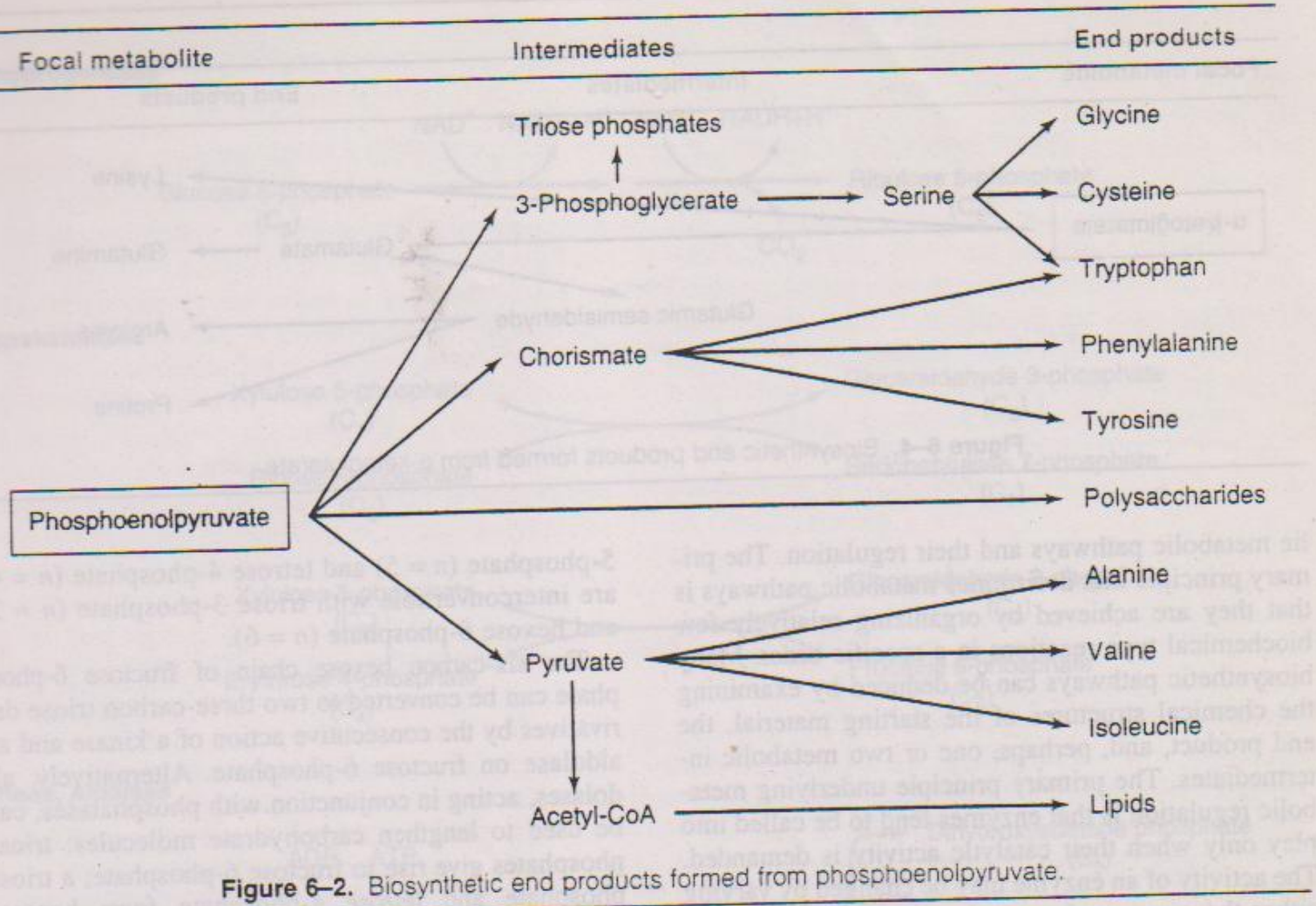
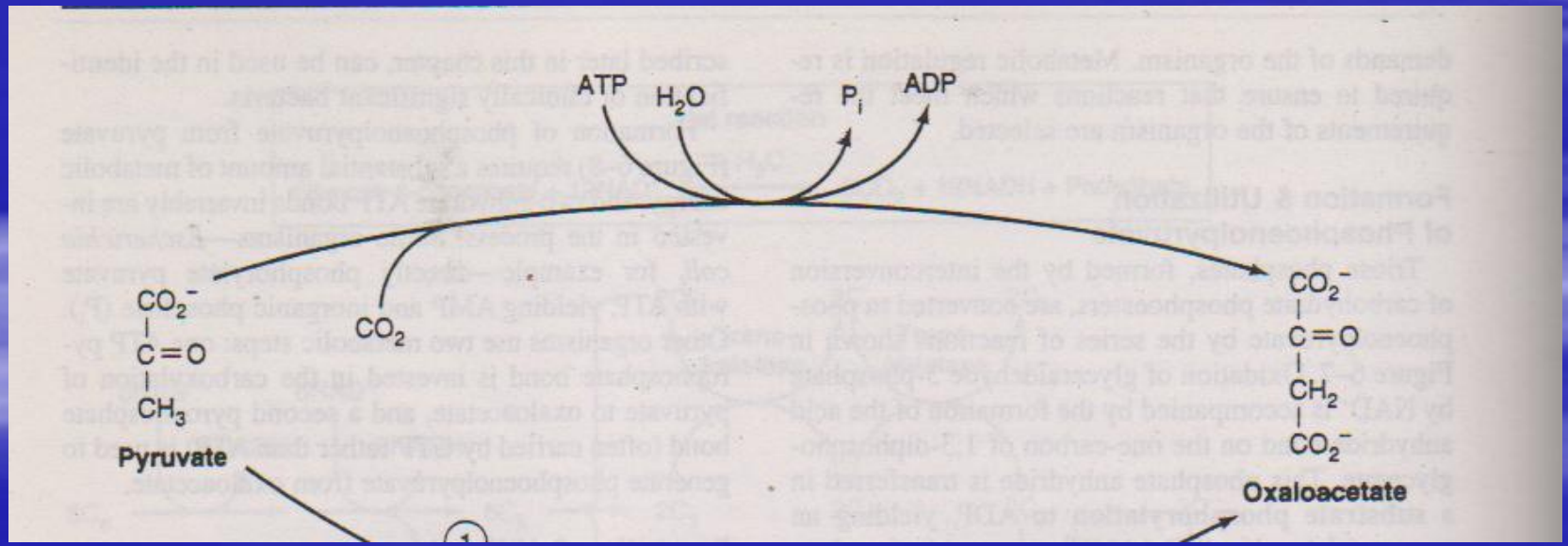


Figure 6–2. Biosynthetic end products formed from phosphoenolpyruvate.

3- oxaloacetate:

- it is formed by the carboxylation of pyrovate



- Biosynthetic end products formed from oxaloacetate:
- the intermediate compound is Aspartate and the end products are

Amino acids, enzymes and nucleic acids.

biosynthesis is balanced: All of the components re-

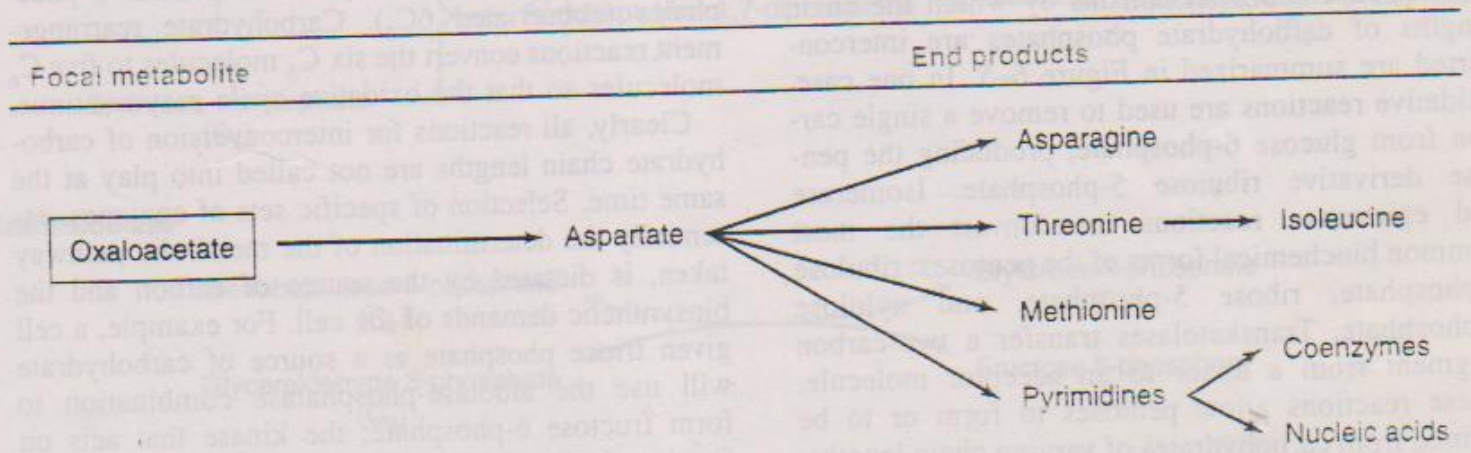


Figure 6–3. Biosynthetic end products formed from oxaloacetate. The end products aspartate, threonine, and pyrimidines serve as intermediates in the synthesis of additional compounds.

4-Alpha-ketoglutarate

- It is one of the Krebs cycle intermediates, conversion of pyrovate to 4-Alph-ketoglutarate requires two metabolic pathways :
- in one way oxaloacetate is formed by carboxylation of pyrovate or Phosphoenolpyrovate and in the other , pyrovate is oxidized to Acetyl Co A. The synthesis of oxaloacetate is balanced with Acetyl Co A production . Condensation of Oxaloacetate with Acetyl Co A
- yields citrate. Isomerization of citrate molecule produces isocitrate, which is oxidatively decarboxylated to alpha keto glutarate.

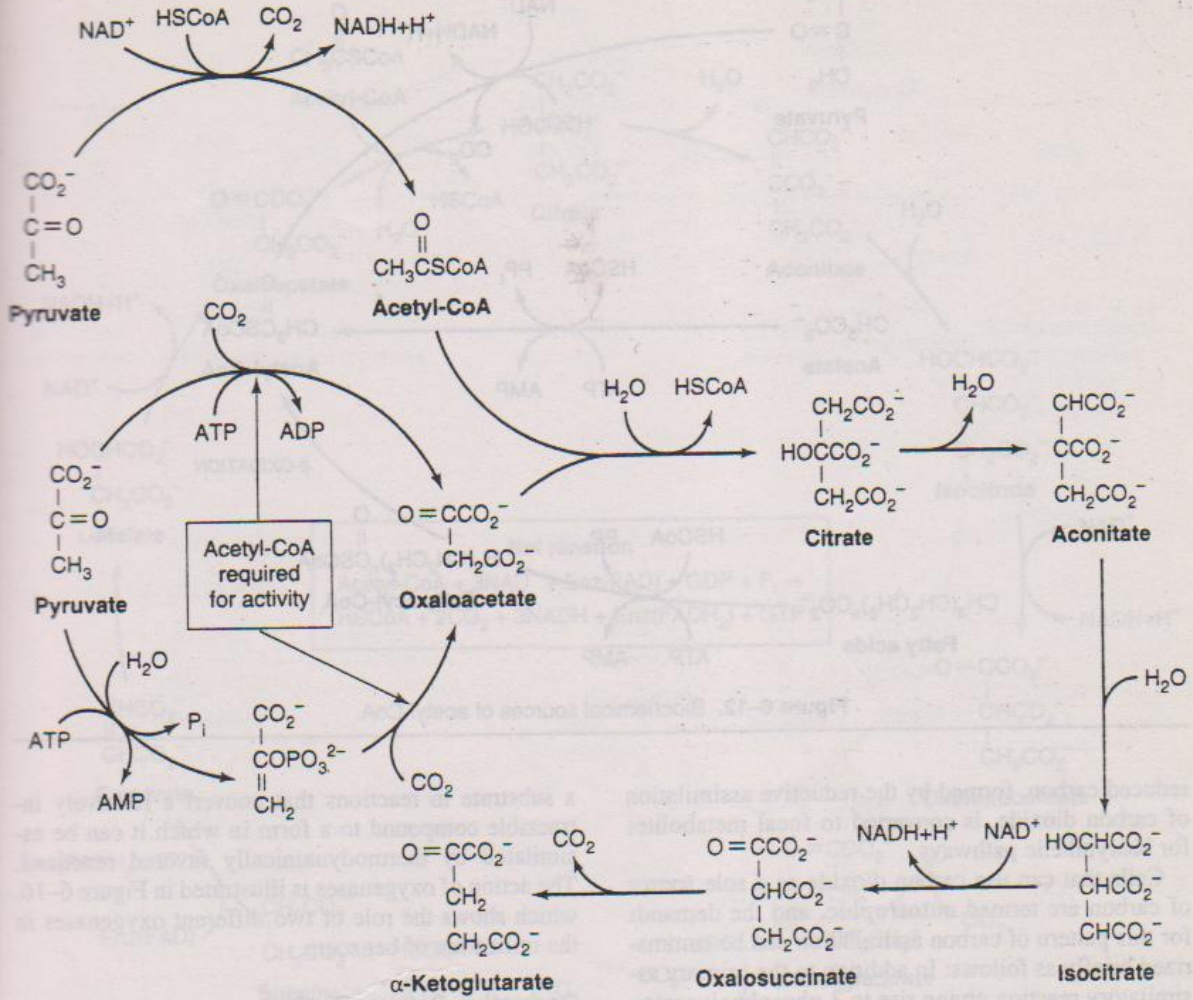


Figure 6–11. Conversion of pyruvate to α-ketoglutarate. Pyruvate is converted to α-ketoglutarate by a branched biosynthetic pathway. In one branch, pyruvate is oxidized to acetyl-CoA; in the other, pyruvate is carboxylated to oxaloacetate. As noted in Figure 6–9, the latter can proceed through either of two mechanisms.

Biosynthetic end products formed from Alpha-Ketoglutarate

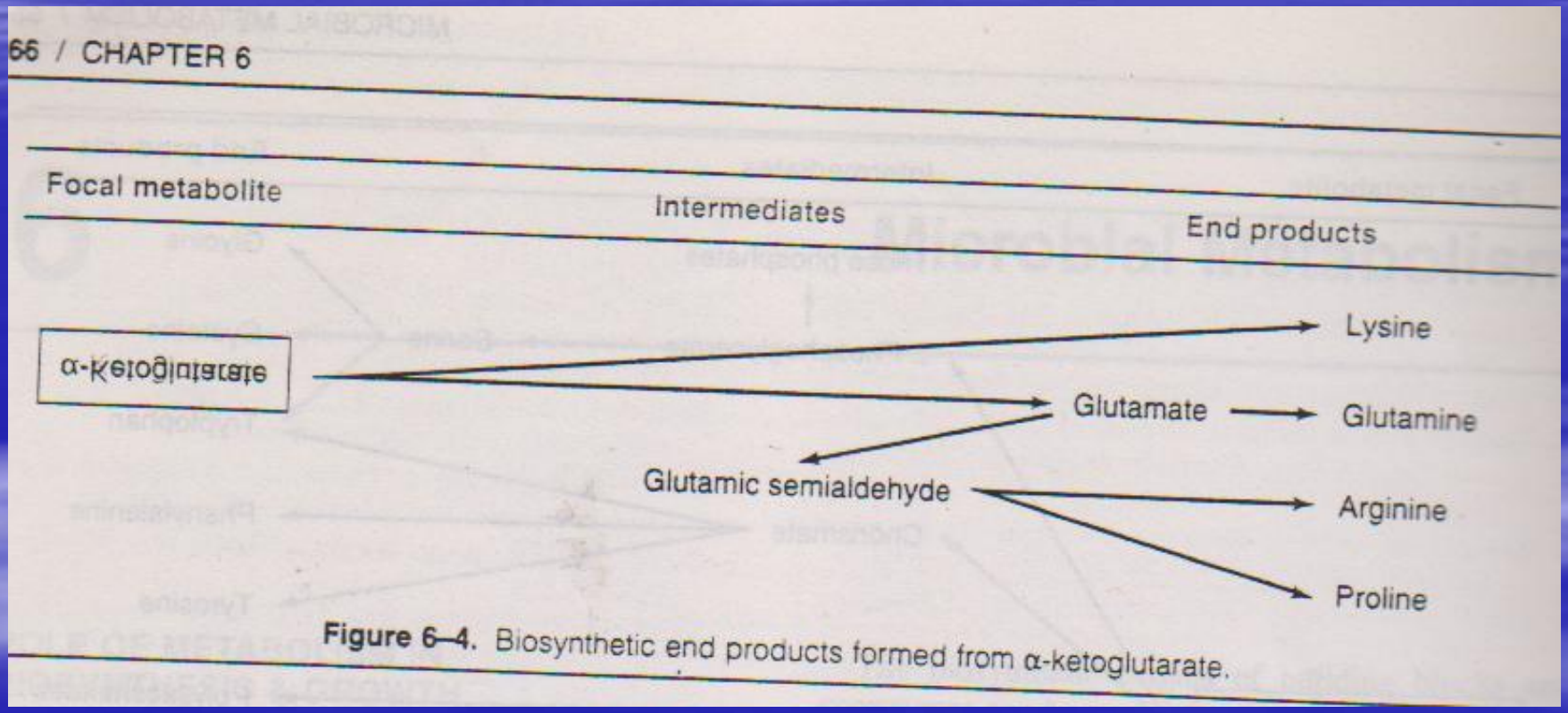


Figure 6–4. Biosynthetic end products formed from α-ketoglutarate.

- In Calvin cycle CO_2 and Ribulose diphopsphate combine to form molecules of 3- phosphoglycerate which can enters assimilation pathways to carbohydrate (pentose phosphate , Fructose 6 phosphate) and this pentose sugar can shifts into pyrovate .

metabolites.

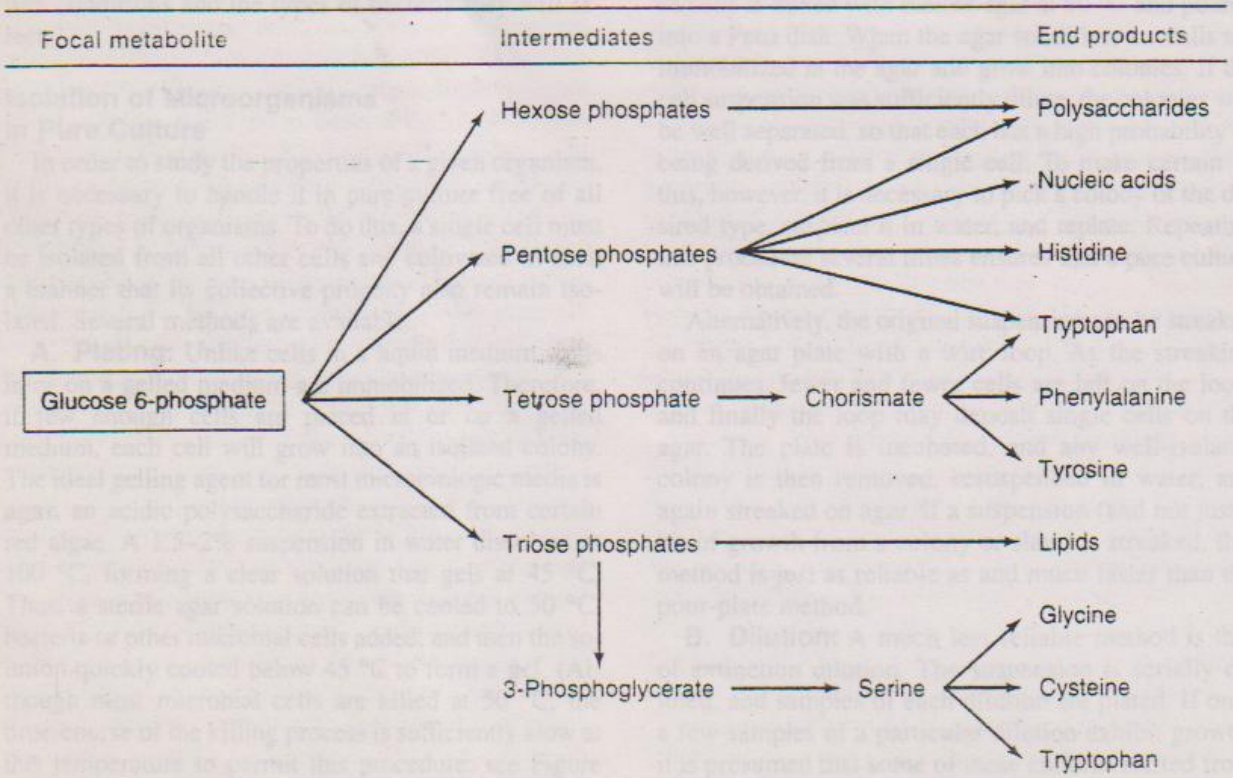


Figure 6–1. Biosynthetic end products formed from glucose 6-phosphate. Carbohydrate phosphate esters of varying chain length serve as intermediates in the biosynthetic pathways.

Growth with acetate:

- Acetate is metabolized via acetyl Co A , and many organisms possess the ability to form acetyl Co A from different metabolites as shown in his figure:

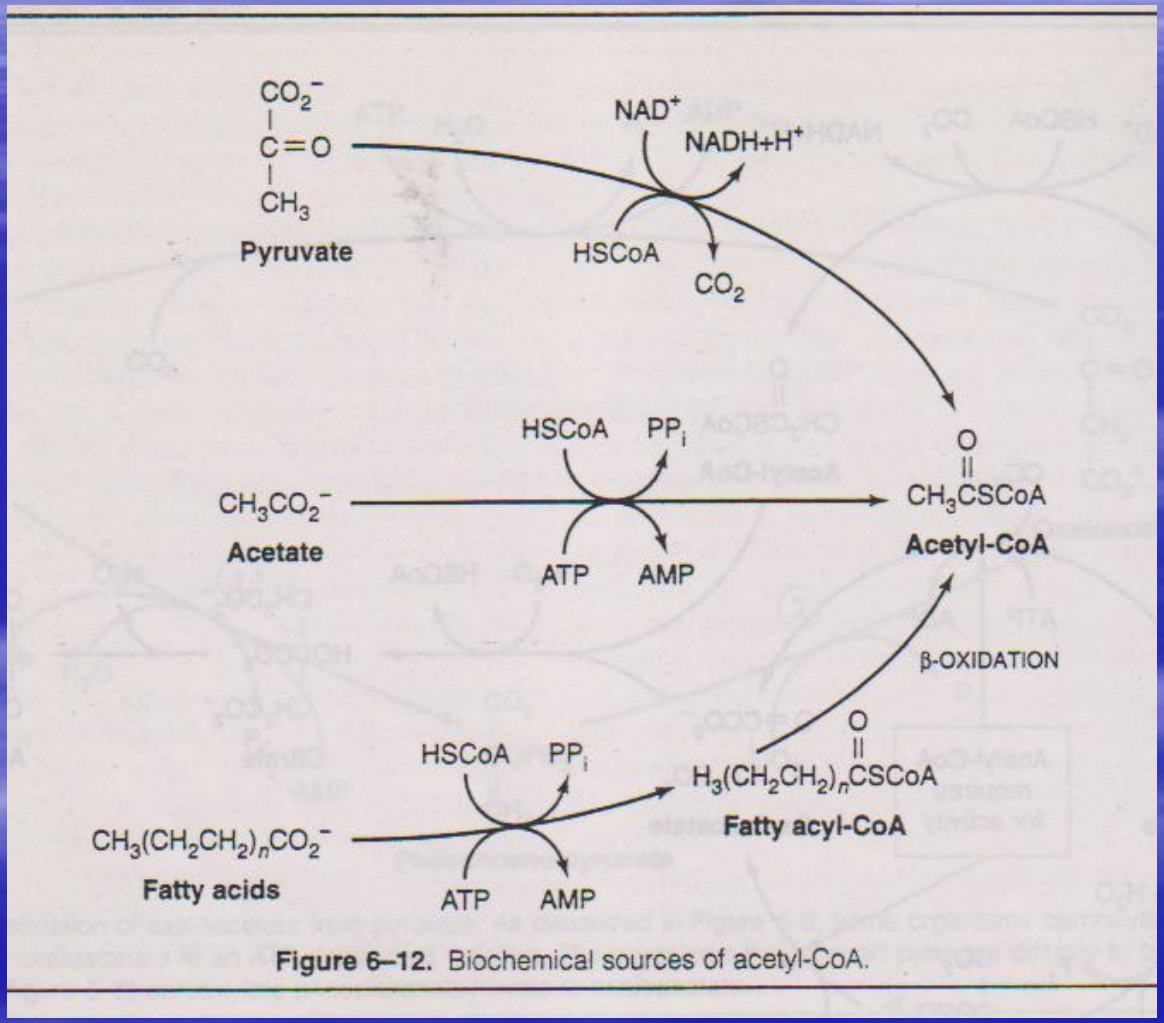


Figure 6–12. Biochemical sources of acetyl-CoA.

Growth with CO_2

- **The Calvin cycle**

  Like plants and algae some microbial types can use CO_2 as a sole source of Carbon. In most of theses microbes the primary route of carbon assimilation route is via the Calvin cycle.

- Acetyl Co A is used in the biosynthesis of Alpha- Ketoglutarate
- And in most organisms respiration , the Acetyl fragment in Co A is oxidized to CO_2 through Krebs cycle.

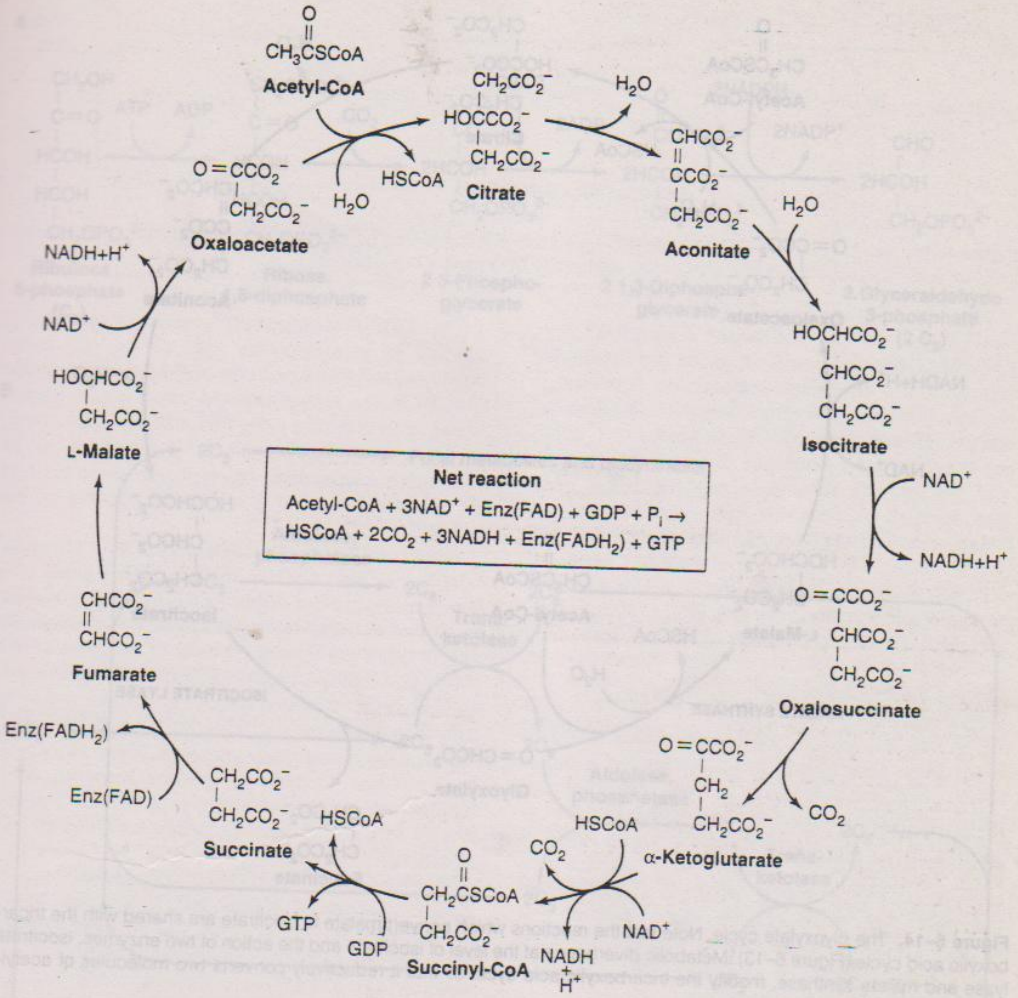


Figure 6–13. The tricarboxylic acid cycle. There are four oxidative steps, three giving rise to NADH and one giving rise to a reduced flavoprotein, $Enz(FADH_2)$. The cycle can continue only if electron acceptors are available to oxidize the NADH and reduced flavoprotein.

Synthesis of bacterial cell wall

synthesis of cell wall peptide glycan:

Gram positive bacterial cell wall structure is well defined in this diagram .

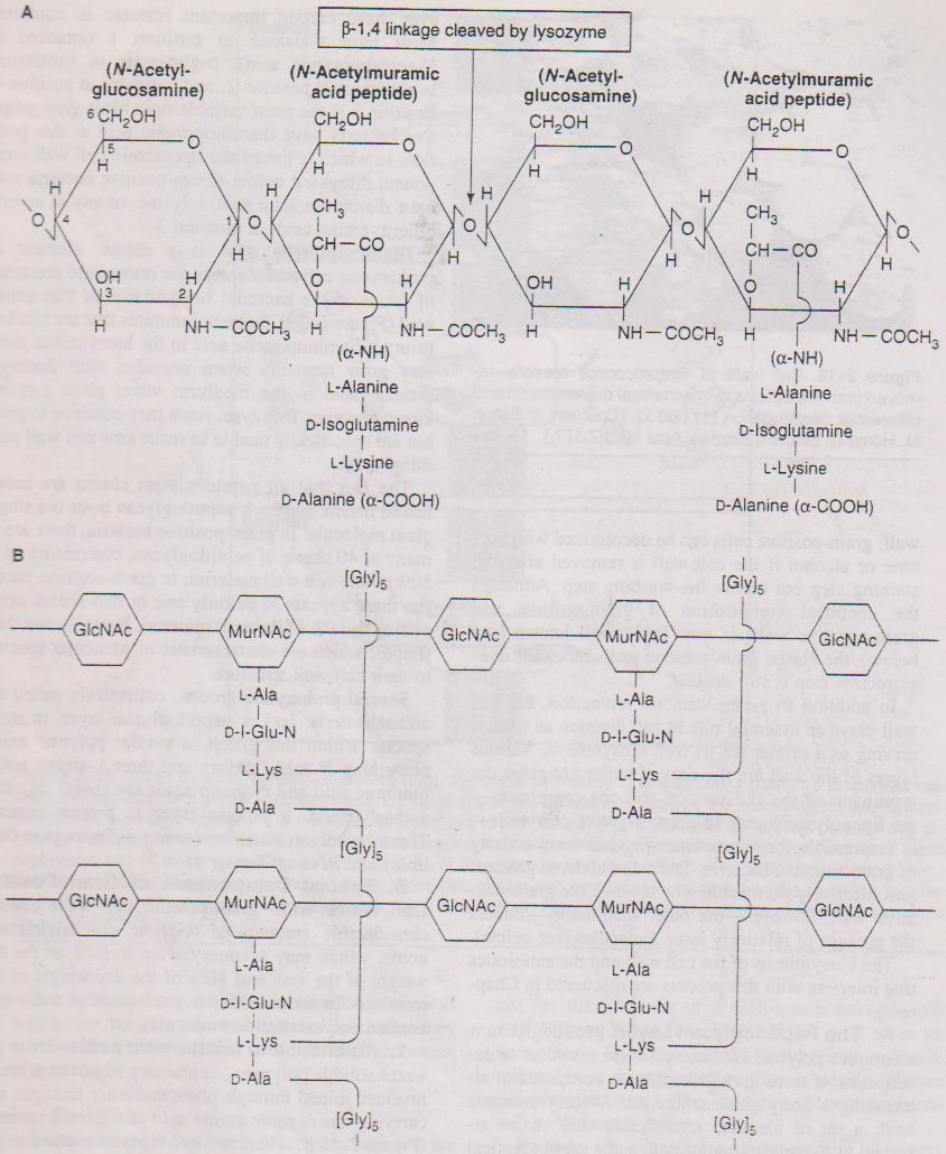


Figure 2–17. ***A:*** A segment of the peptidoglycan of *Staphylococcus aureus*. The backbone of the polymer consists of alternating subunits of *N*-acetylglucosamine and *N*-acetylmuramic acid connected by β-1,4 linkages. The muramic acid

Synthesis of peptidoglycan

- Cell wall peptidoglycan begins with stepwise synthesis in cytoplasm of UDP – N-Acetyl muramic acid pentapeptide.
- N-Acetyl glucose amine is first attached to UDP then converted to UDP – N-Acetyl muramic acid by condensation with phosphoenol pyrovate and reduction .

Binding to pentapeptide

- **The amino acids of the pentapeptide are sequentially added to N - acetylmuramicacid, (L Alanine, D Glutamine, L. Lysine. D. Alanine, D. Alanine) .**

Binding to the cell membrane

- **The UDP-N- Acetylmuramic acid – pentapeptide is bound to the cell membrane (through bactophenol , a lipid of bacterial cell membrane) and receive a molecule of N-Acetyl glucose amine from UDP.**

Transpeptidation

- **Final cross-linking is accomplished by a Transpeptidation reaction in which the free amino group of a pentaglycine residue displaces the terminal D-Alanine residue of a neighboring pentapeptide .**

PBP

- **Transpeptidation is catalyzed by one of a set of enzymes called penicillin binding proteins (PBPs) these PBPs bind penicillin and other betalactam antibiotics covalently due to structural similarity between these betalactams and pentapeptide precursors .**

Steps of bacterial cell wall biosynthesis

- so we can conclude the steps of cell wall peptidoglycan biosynthesis into the following steps:
- 1- synthesis of water soluble complex
- 2-attachment to the cell membrane
- 3- formation of linear polymer out side cell membrane .
- 4- Cross linkage of polymers .
- 5- Transpeptidation.

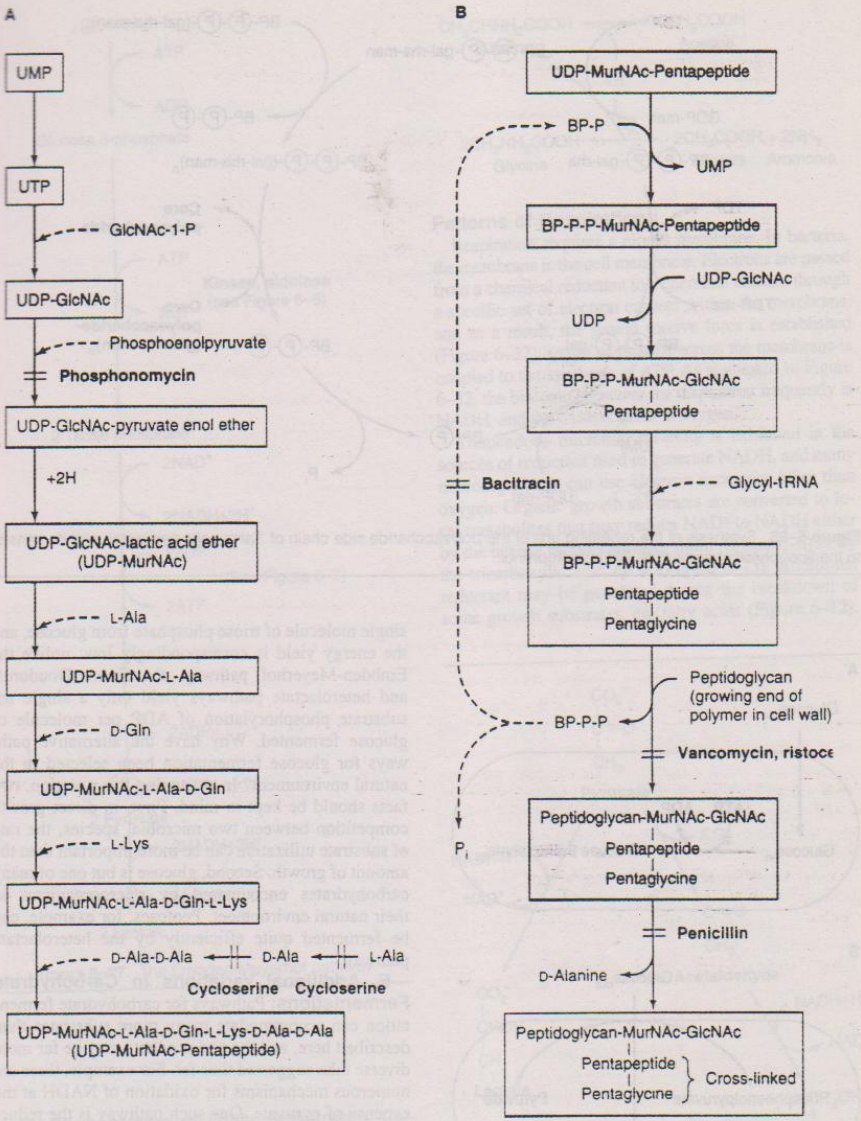
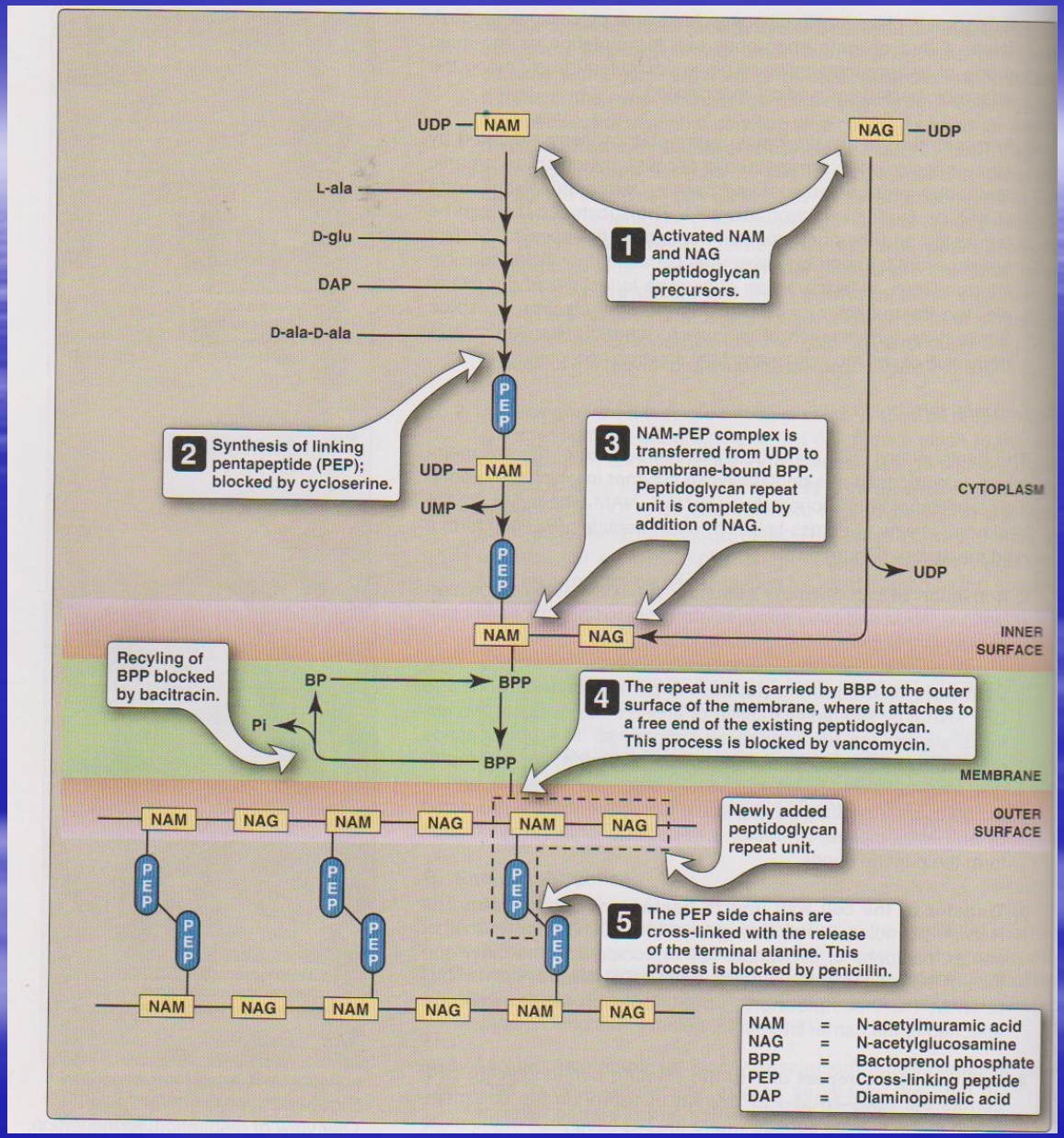


Figure 6–24. The biosynthesis of cell wall peptidoglycan, showing the sites of action of six antibiotics. BP = bactoprenol; MurNAc = *N*-acetylmuramic acid; GlcNAc = *N*-acetylglucosamine. ***A:*** Synthesis of UDP-acetylmuramic acid-pentapeptide. ***B:*** Synthesis of peptidoglycan from UDP-acetylmuramic acid-pentapeptide, UDP-*N*-acetylglucosamine, and glycyl residues. (See Figure 2–17 for structure of peptidoglycan.)

cell walls biosynthesis steps and selectivity of some antibiotics

- The biosynthesis of the bacterial cell wall is of a particular importance in medicine as it provides a basis for selective antibacterial action of several chemotherapeutic agents. Any compound that inhibits any step in the biosynthesis of peptidoglycan causes the wall of bacterial cell to be weakened and the cell lyses .

- The sites of action of several antibiotics on the steps of cell wall synthesis are sown in the following figure:


UDP — NAM
NAG — UDP
L-ala
D-glu
DAP
D-ala-D-ala
1 Activated NAM and NAG peptidoglycan precursors.
2 Synthesis of linking pentapeptide (PEP); blocked by cycloserine.
3 NAM-PEP complex is transferred from UDP to membrane-bound BPP. Peptidoglycan repeat unit is completed by addition of NAG.
UDP — NAM
UMP
CYTOPLASM
UDP
NAM
NAG
INNER SURFACE
Recyling of BPP blocked by bacitracin.
BP
BPP
Pi
4 The repeat unit is carried by BBP to the outer surface of the membrane, where it attaches to a free end of the existing peptidoglycan. This process is blocked by vancomycin.
BPP
MEMBRANE
OUTER SURFACE
Newly added peptidoglycan repeat unit.
5 The PEP side chains are cross-linked with the release of the terminal alanine. This process is blocked by penicillin.
NAM = N-acetylmuramic acid
NAG = N-acetylglucosamine
BPP = Bactoprenol phosphate
PEP = Cross-linking peptide
DAP = Diaminopimelic acid