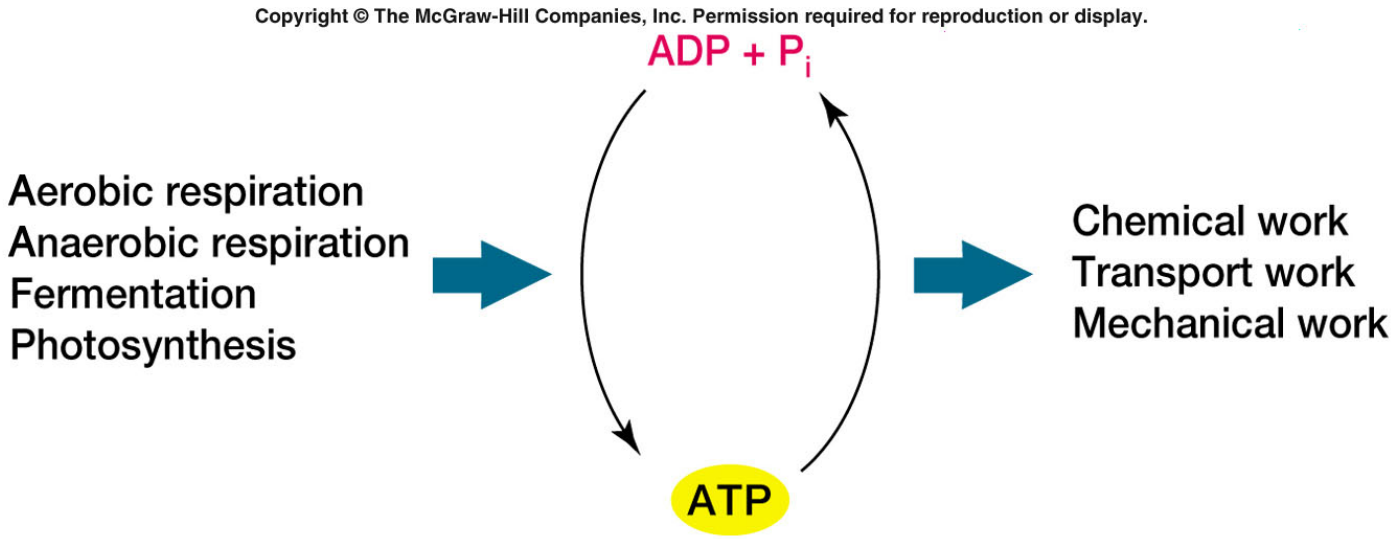
Biosynthetic Pathways for Metabolic Products of Microorganisms

1. The Nature of Metabolic Pathways

In order to be able to manipulate microorganisms to produce maximally materials of economic importance to humans, but at minimal costs, it is important that the physiology of the organisms be understood as much as is possible.

Microbial Metabolism



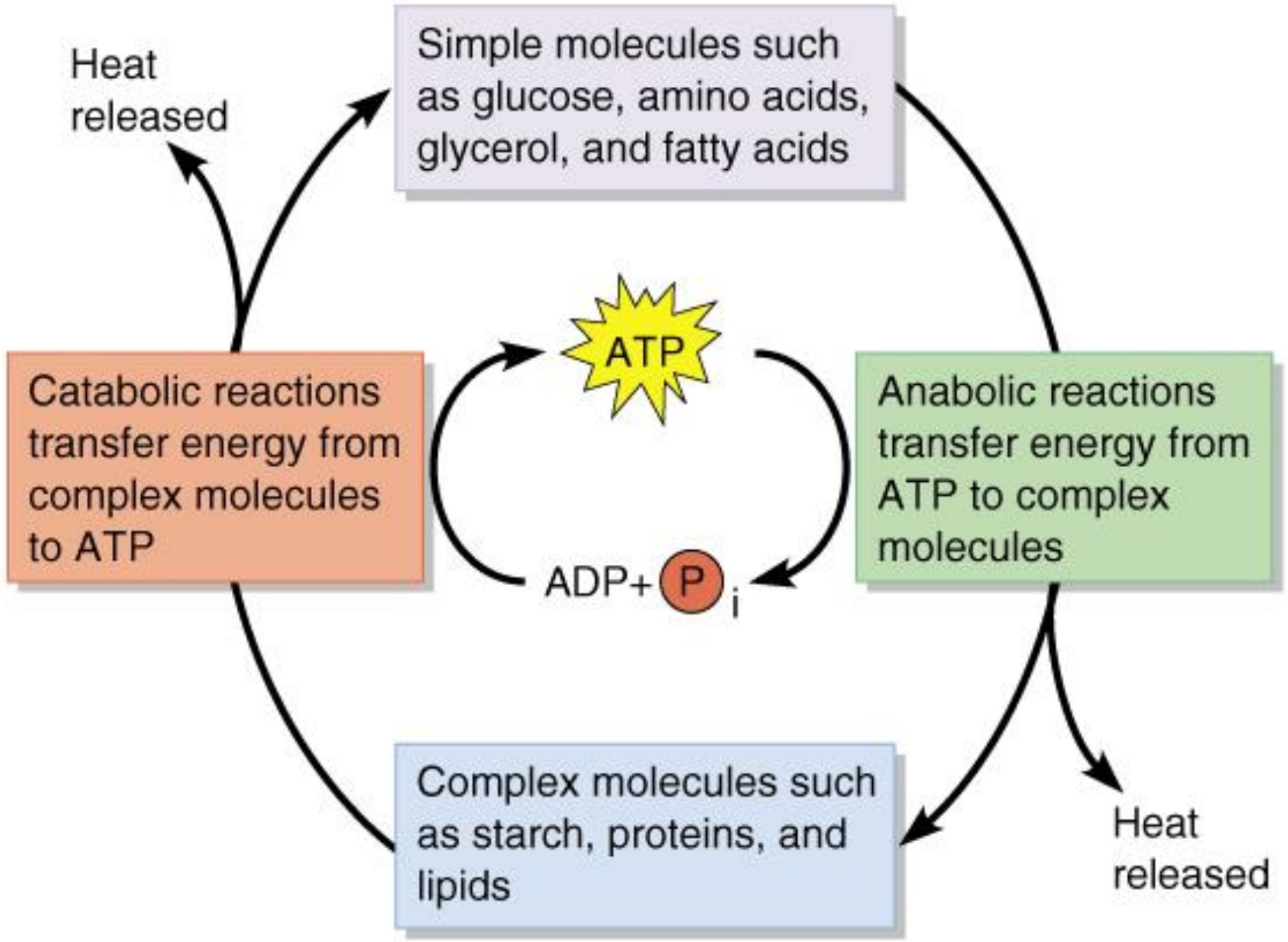
- **Metabolic pathway** is the series of chemical reactions involved in converting a chemical (or a metabolite) in the organism into a final product.

- When the reactions lead to the formation of a more complex substance, that particular form of metabolism is known as **anabolism/anabolic pathway**.

- When the series of reactions lead to less complex compounds the metabolism is described as **catabolism**.

- The compounds involved in a metabolic pathway are called **intermediates**.

- The final product is known as **the end-product.**


Heat released
Simple molecules such as glucose, amino acids, glycerol, and fatty acids
ATP
Catabolic reactions transfer energy from complex molecules to ATP
Anabolic reactions transfer energy from ATP to complex molecules
ADP+ P i
Complex molecules such as starch, proteins, and lipids
Heat released

Catabolic reactions

- Catabolic reactions have been mostly studied with glucose.
- Four pathways of glucose breakdown to pyruvic acid (or glycolysis) are currently recognized.
- Catabolic reactions often furnish energy in the form of ATP and other high energy compounds, which are used for biosynthetic reactions.
- A second function of catabolic reactions is to provide the carbon skeleton for biosynthesis.

Anabolic reactions

Anabolic reactions lead to the formation of larger molecules some of which are constituents of the cell.

2. Industrial Microbiological Products as Primary and Secondary Metabolites

Products of industrial microorganisms may be divided into two broad groups.

2.1 Products of Primary Metabolism

2.2 Products of Secondary Metabolism

2.1 Products of Primary Metabolism

- Primary metabolism is the inter-related group of reactions within a microorganism.
- Primary metabolism is the same in all living things and is concerned with the release of energy, and the synthesis of important macromolecules such as proteins, nucleic acids and other cell constituents.
- When primary metabolism is stopped the organism dies.
- Products of primary metabolism are associated with growth and their maximum production occurs in the logarithmic phase of growth in a batch culture.
- Primary catabolic products include ethanol, lactic acid, and butanol while anabolic products include amino-acids, enzymes and nucleic acids.

2.2 Products of Secondary Metabolism

Secondary metabolism, which was first observed in higher plants, has the following characteristics

(i) Secondary metabolism has no apparent function in the organism.

The organism continues to exist if secondary metabolism is blocked but it would die if primary metabolism were stopped.

(ii) Secondary metabolites are produced in response to a restriction in nutrients.

They are therefore produced after the growth phase, at the end of the

logarithmic phase of growth and in the stationary phase

(iii) Secondary metabolism is restricted to some species of plants and microorganisms.

The products of secondary metabolism also appear to be characteristic of the species.

(iv) Secondary metabolites usually have ‘bizarre’ and unusual chemical structures and several closely related metabolites may be produced by the same organism in wild-type strains.

(v) The ability to produce a particular secondary metabolite is easily lost. This phenomenon is known as strain degeneration.

(vi) Owing to the ease of the loss of the ability to synthesize secondary metabolites, particularly when treated with acridine dyes, exposure to high temperature or other treatments known to induce plasmid loss. Secondary metabolite production is believed to be controlled by plasmids.

(vii) The factors which trigger secondary metabolism,the inducers, also trigger morphological changes (morphogenesis) in the organism.

2.2.1 Inducers of Secondary Metabolites

Autoinducers include

1. The ɣ-butyrolactones (butanolides) of the actinomycetes
2. The Nacylhomoserine lactones (HSLs) of Gramnegative bacteria
3. The oligopeptides of Grampositive bacteria
4. B-factor [3'-(1-butylphosphoryl)adenosine] of rifamycin production in *Amycolatopsis mediterrane*.

They function in development, sporulation, light emission, virulence, production of antibiotics, pigments and cyanide, plasmiddriven conjugation and competence for genetic transformation.

Example:

Actinomycete fermentations is the inducing effect of endogenous -butyrolactones, e.g. Afactor (2-S-isocapryloyl-3R-hydroxymethyl--butyrolactone).

A-factor induces both morphological and chemical differentiation in *Streptomyces griseus* and *Streptomyces bikiniensis*, bringing on formation of aerial mycelia, conidia, streptomycin synthases and streptomycin.

Many other actinomycetes produce A-factor, or related-butyrolactones, which differ in the length of the side-chain. In those strains which produce antibiotics other than streptomycin, the -butyrolactones induce formation of the particular antibiotics that are produced, as well as morphological differentiation.

2.2.2 Secondary metabolic products of microorganism

Microbial secondary metabolites include

1. Antibiotics,
2. Pigments,
3. Toxins,
4. Effectors of ecological competition and symbiosis,
5. Pheromones,
6. Enzyme inhibitors,
7. Immunomodulating agents,
8. Receptor antagonists and agonists, pesticides,
9. Antitumor agents and
10. Growth promoters of animals and plants, including gibbrellic acid, antitumor agents, alkaloids such as ergometrine, a wide variety of other drugs, toxins and useful materials such as the plant growth substance, gibberellic acid.

- Microbial secondary metabolites have a major effect on the health, nutrition, and economics of our society.
- They often have unusual structures and their formation is regulated by nutrients, growth rate, feedback control, enzyme inactivation, and enzyme induction.
- Regulation is influenced by unique low molecular mass compounds, transfer RNA, sigma factors, and gene products formed during post-exponential development.
- The synthases of secondary metabolism are often coded for by clustered genes on chromosomal DNA and infrequently on plasmid DNA.
- Thousands of secondary metabolites of widely different chemical groups and physiological effects on humans have been found.

3.Trophophase-Idiophase Relationships in the Production of Secondary Products

From studies on *Penicillium urticae* the terms trophophase and idiophase were introduced to distinguish the two phases in the growth of organisms producing secondary metabolites.

The trophophase (Greek, tropho = nutrient) is the feeding phase during which primary metabolism occurs. In a batch culture this would be in the logarithmic phase of the growth curve.

The trophophase is the idio-phase (Greek, idio = peculiar) during which secondary metabolites peculiar to a given organism are synthesized. Secondary synthesis occurs in the late logarithmic, and in the stationary, phase. It has been suggested that secondary metabolites be described as ‘idiolites’ to distinguish them from primary metabolites.

4. Role of Secondary Metabolites in the Physiology of Organisms Producing Them

(4.1) The competition hypothesis:

In this theory which refers to antibiotics specifically, secondary metabolites (antibiotics) enable the producing organism to withstand competition for food from other soil organisms.

(4.2) The maintenance hypothesis:

Secondary metabolism usually occurs with the exhaustion of a vital nutrient such as glucose. It's selective advantage is that it serves to maintain mechanisms essential to cell multiplication when that cell is no longer possible. Thus by forming secondary enzymes, the enzymes of primary metabolism would be destroyed. Therefore, the secondary metabolite itself is not important; what is important is the pathway of producing it.

(4.3) **The unbalanced growth hypothesis:**

Similar to the maintenance theory, this hypothesis states that control mechanisms in some organisms are too weak to prevent the over synthesis of some primary metabolites. These primary metabolites are converted into secondary metabolites that are excreted from the cell. If they are not so converted they would lead to the death of the organism.

(4.4) **The detoxification hypothesis**:

This hypothesis states that molecules accumulated in the cell are detoxified to yield antibiotics. This is consistent with the observation that the penicillin precursor penicillanic acid is more toxic to Penicillium chrysogenum than benzyl penicillin. Nevertheless not many toxic precursors of antibiotics have been observed.

(4.5) **The regulatory hypothesis**:

Secondary metabolite production is known to be associated with morphological differentiation in producing organisms. In the fungus *Neurospora crassa*, carotenoids are produced during sporulation. In *Cephalospoium acremonium*, cephalosporin C is produced during the idiophase when arthrospores are produced.

In this theory the production of secondary metabolites is necessary to regulate some morphological changes in the organism. It could of course be that some external mechanism triggers off secondary metabolite production as well as the morphological change.

(4.6) **The hypothesis of secondary metabolism as the expression of evolutionary reactions:**

In this hypothesis, it is important to bear in mind that both primary and secondary metabolism are controlled by genes carried by the organism. Any genes not required are lost.

According to this hypothesis, secondary metabolism is a mixed bag of biochemical reactions, undergoing tests for possible incorporation into the cell's armory of primary reactions. Any reaction in the mixed bag which favorably affects any one of the primary processes, thereby fitting the organism better to survive in its environment, becomes incorporated as part of primary metabolism.

According to this hypothesis, the antibiotic properties of some secondary metabolites are incidental and not a design to protect the microorganisms. This hypothesis implies that secondary metabolism must occur in all microorganisms since evolution is a continuing process.

5. Pathways for the Synthesis of Primary and Secondary Metabolites of Industrial Importance

The main source of carbon and energy in industrial media is carbohydrates. In recent times hydrocarbons have been used. The catabolism of these compounds supply the carbon skeletons for the synthesis of primary as well as for secondary metabolites.

5.1 Catabolism of Carbohydrates

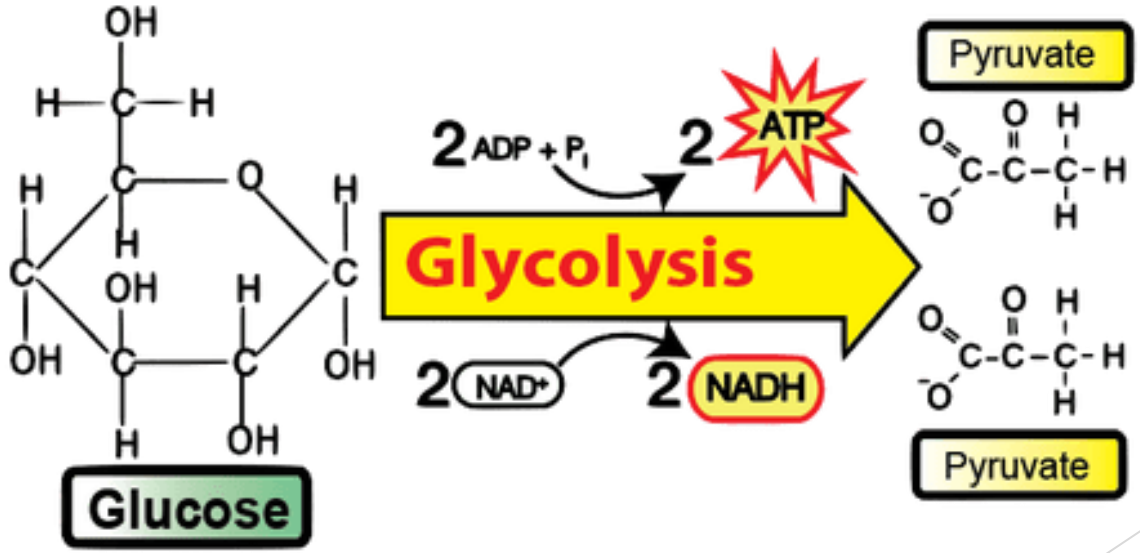
Four pathways for the catabolism of carbohydrates (glucose) up to pyruvic acid are :

1. The Embden-Meyerhof-Parmas
2. The Pentose Phosphate Pathways
3. The Entner Duodoroff pathway
4. The Phosphoketolase pathway

All four pathways exist in bacteria, actinomycets and fungi, including yeasts.

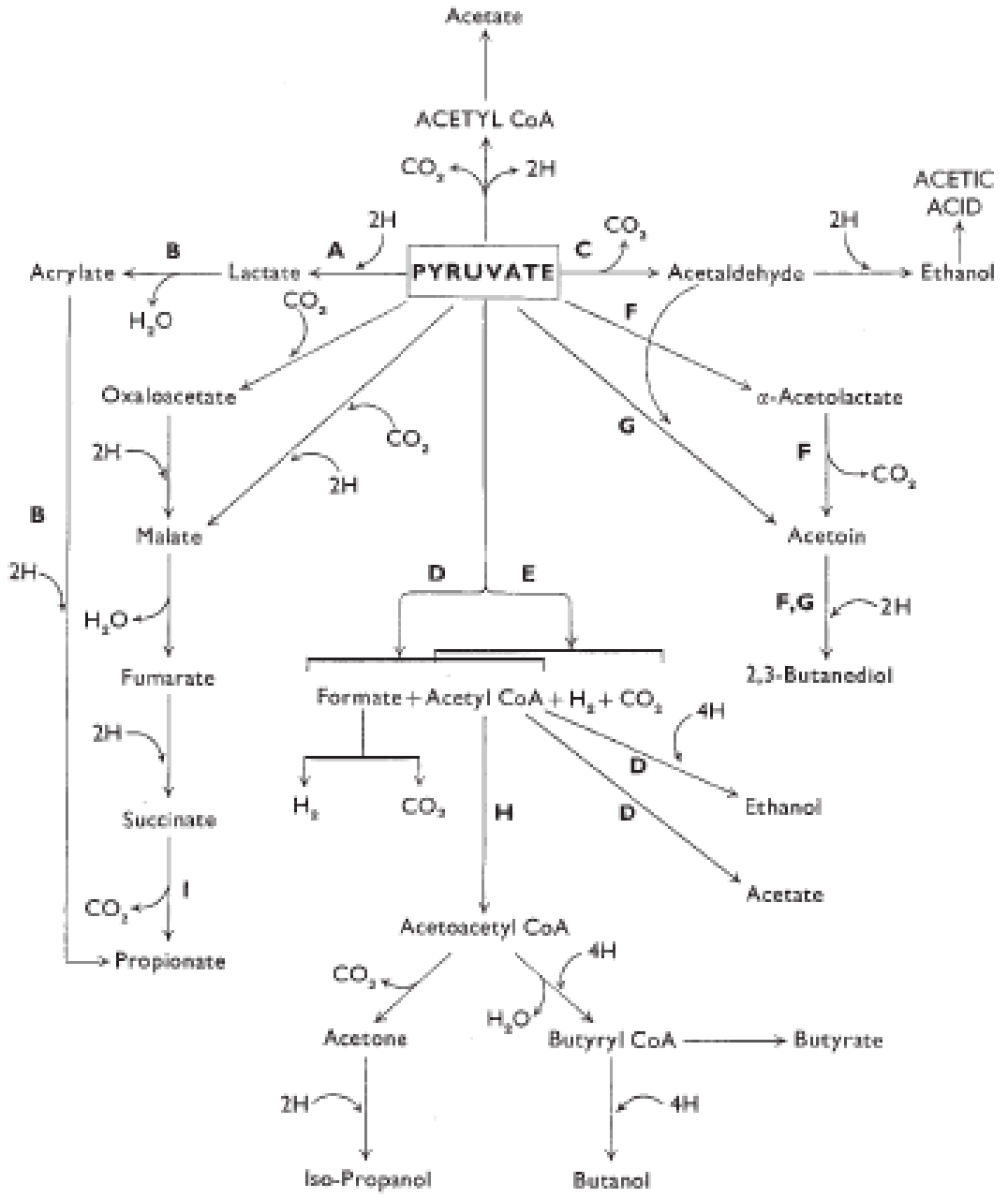
1. The Embden-Meyerhof-Parnas (EMP Pathways):

- The net effect of this pathway is to reduce glucose (C6) to pyruvate (C3).
- The system can operate under both aerobic and anaerobic conditions.
- Under aerobic conditions it usually functions with the tricarboxylic acid cycle which can oxidize pyruvate to CO_2 and H_2O.
- Under anaerobic conditions, pyruvate is fermented to a wide range of fermentation products, many of which are of industrial importance



Products of the Fermentation of Pyruvate by Different Microorganisms

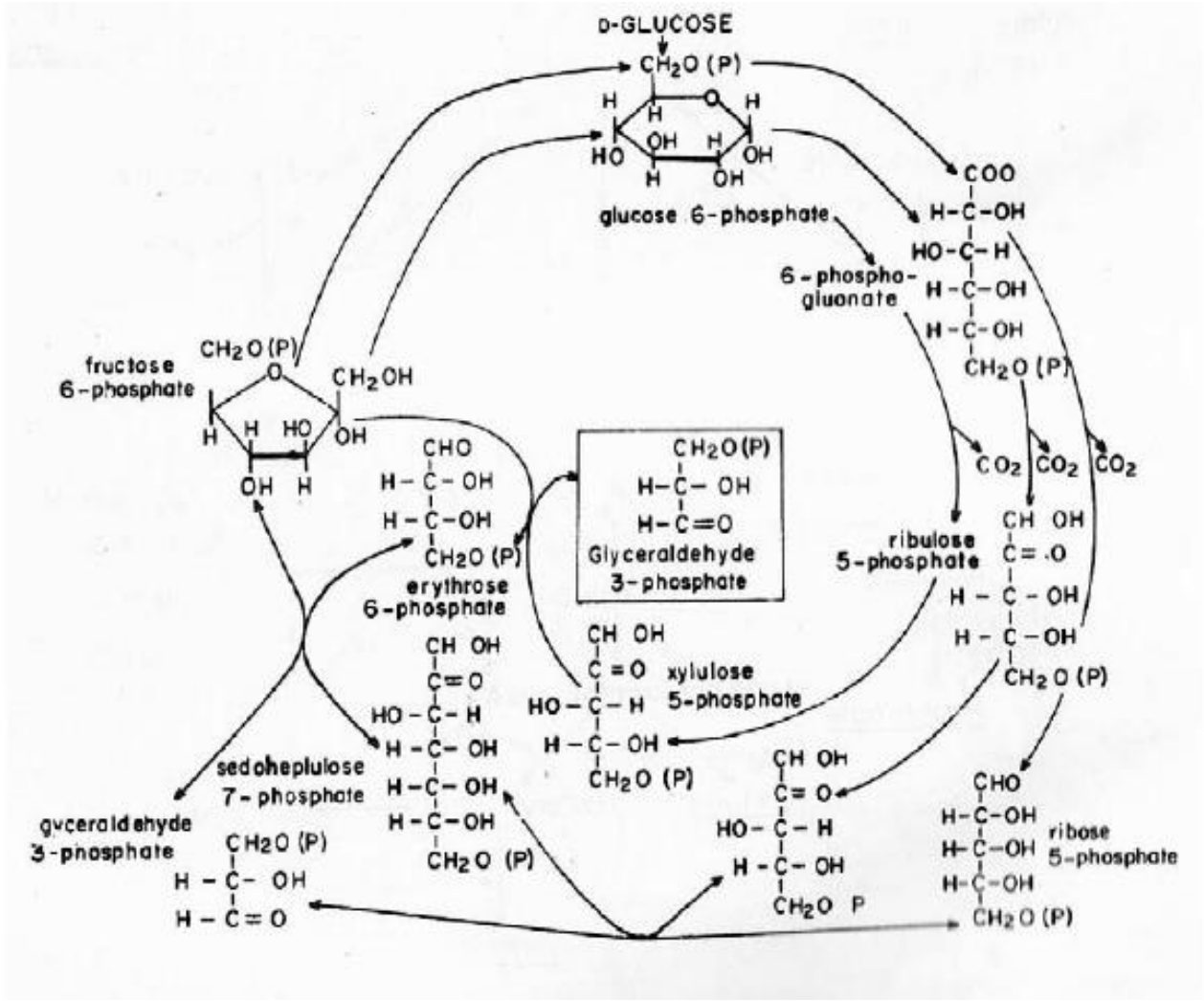
A	(End product, Lactate)	Lactic acid bacteria
B	(End product, Acrylate)	*Clostridium propinicum*
C	(End product, Ethanol)	Yeasts, *Acetobacter, Zymomonas*
D	(Formic acid, H_2, CO_2, Ethanol)	*Enterobacteriaceae*
E	(H_2, CO_2, Ethanol)	Clostridia
F	(Acetoin, 2-3 Butanediol)	Aerobacter
G	(Acetoin, 2-3 Butanediol)	Yeasts
H	(Acetone, Isoprpanol, Acetone	Clostridia (butyric acid)
I	(Propioninate)	Propionic acid bacteria

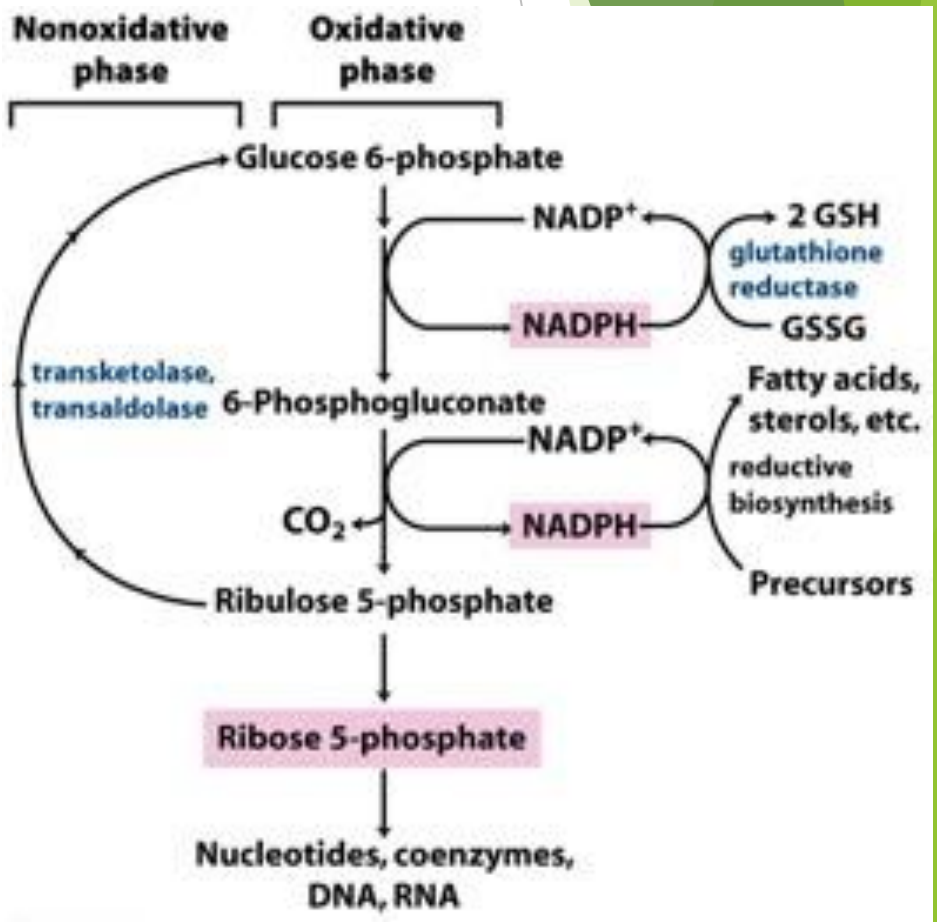


2. *The pentose Phosphate Pathway (PP)*:

- This is also known as the Hexose Monophosphate Pathway **(HMP**) or the phosphogluconate pathway.
- While the **EMP** pathway provides pyruvate, a C3 compound, as its end product, there is no
- end product in the PP pathway.
- Instead it provides a pool of triose (C3) pentose (C5), hexose (C6) and heptose (C7) phosphates. The primary purpose of the PP pathway, however, appears to be to generate energy in the form of **NADPA2** for biosynthetic and other purposes and pentose phosphates for nucleotide synthesis

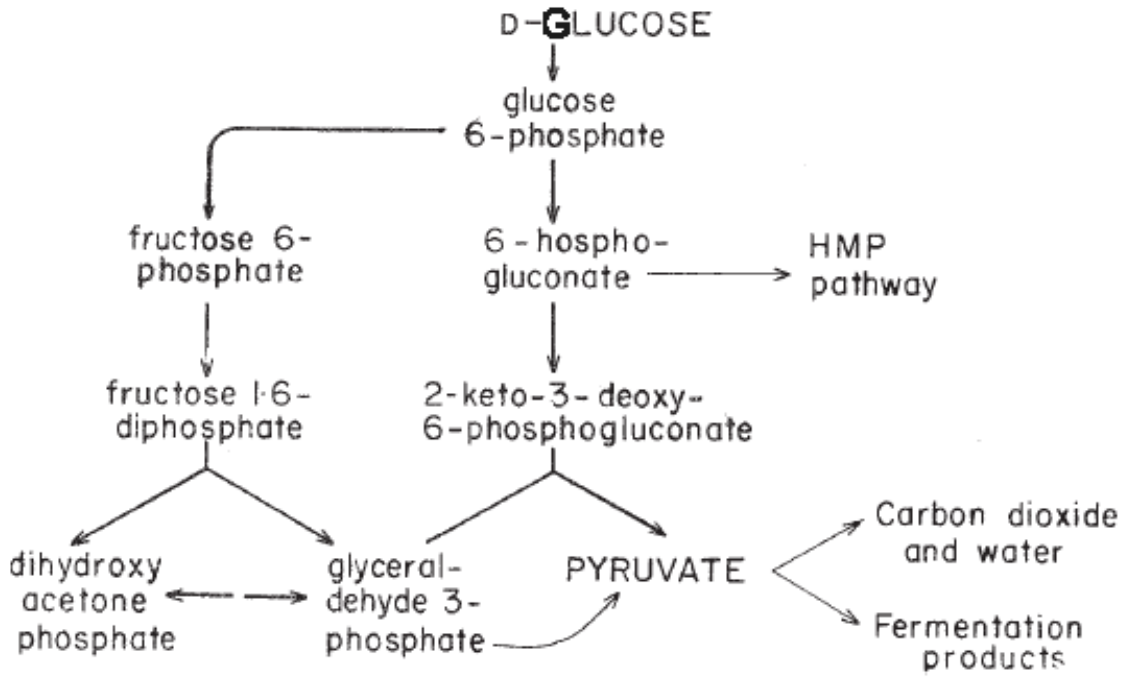
The pentose Phosphate Pathway (PP):





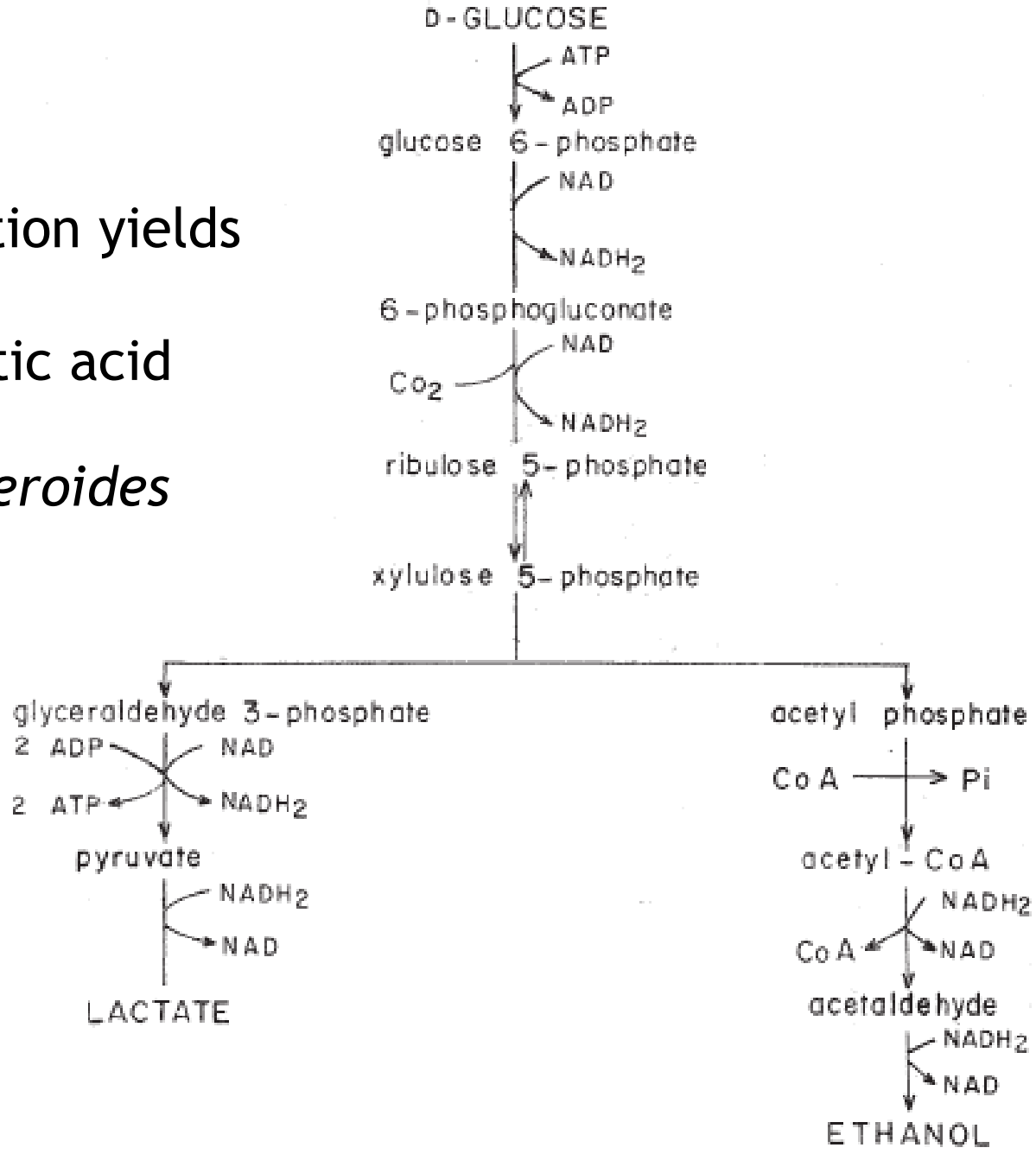
3. *The Entner-Duodoroff Pathway (ED)*:

- The pathway is restricted to a few bacteria especially *Pseudomonas*, but it is also carried out by some fungi.
- It is used by some organisms in the enaerobic breakdown of glucose and by others only in gluconate metabolism



4. *The Phosphoketolase Pathway*:

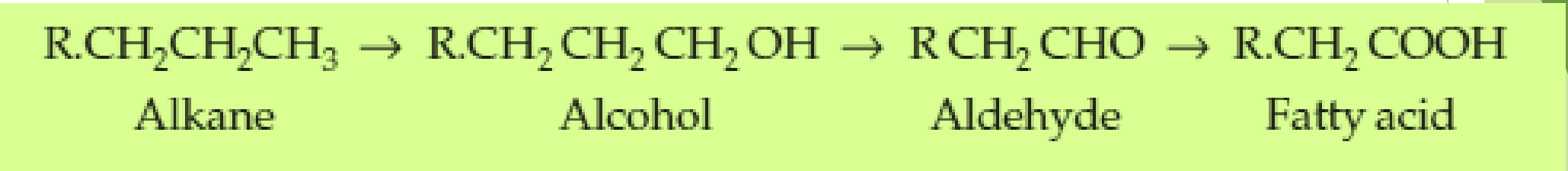
- In some bacteria glucose fermentation yields lactic acid, ethanol and CO_2.
- Pentoses are also fermented to lactic acid and acetic acid.
- An example is *Leuconostoc mesenteroides*



5.2 The Catabolism of Hydrocarbons

(1) Alkanes:

Alkanes are saturated hydrocarbons that have the general formula C2 Hn+2. When the alkanes are utilized, the terminal methyl group is usually oxidized to the corresponding primary alcohol thus:



The alcohol is then oxidized to a fatty acid, which then forms as ester with coenzyme A. Thereafter, it is involved in a series of -oxidations which lead to the step-wise cleaving off of acetyl coenzyme A which is then further metabolized in the Tricarboxylic Acid Cycle

(2) ***Alkenes***:

The alkenes are unsaturated hydrocarbons and contain many double bonds. Alkenes may be oxidized at the terminal methyl group as shown earlier for alkanes. They may also be oxidized at the double bond at the opposite end of the molecule by molecular oxygen given rise to a diol (an alcohol with two –OH groups). Thereafter, they are converted to fatty acid and utilized as indicated above.

6.Carbon Pathways for the Formation of Industrial Products Derived from Primary Metabolism

The broad flow of carbon in the formation of industrial products resulting from primary metabolism may be examined under two headings:

(i) Catabolic products resulting from fermentation of pyruvic acid

(ii) Anabolic products

6.1 Catabolic Products

- Industrial products which are catabolic products formed from carbohydrate fermentation are derived from pyruvic acid produced via the EMP, PP, or ED pathway.
- Those of importance are ethanol, acetic acid, 2, 3-butanediol, butanol, acetone and lactic acid.
- The nature of the products not only broadly depends on the species of organisms used but also on the prevailing environmental conditions such as pH, temperature,aeration, etc.

6.2 Anabolic Products

- Anabolic primary metabolites of industrial interest include amino acids, enzymes, citric acid, and nucleic acids.

7.Carbon Pathways for the Formation of Products of Microbial Secondary Metabolism of Industrial Importance

The unifying features of the synthesis of secondary metabolic products by microorganisms can be summarized thus:

1. Conversion of a normal substrate into important intermediates of general metabolism;
2. The assembly of these intermediates in an unusual way, by means of a combination of standard general mechanisms with a selection from a relatively small number of special mechanism;
3. These special mechanisms while being peculiar to secondary metabolism are not unrelated to general or primary mechanism;
4. The synthetic activity of secondary metabolism appears in response to conditions favorable for cell multiplication

From the above, it becomes clear that although secondary metabolites are diverse in their intrinsic chemical nature as well as in the organism which produce them, they use only a few biosynthetic pathways which are related to, and use the intermediates of, the primary metabolic pathways. Based on the broad flow of carbon through primary metabolites to secondary metabolites,

The secondary metabolites may then be classified according to the following six metabolic pathways.

(1) Secondary products derived from the intact glucose skeleton:

The carbon skeleton of glucose is incorporated unaltered in many antibiotics and other secondary metabolites. The entire basic structure of the secondary product may be derived from glucose as in streptomycin or it may form the glycoside molecule to be combined with a non-sugar (aglycone portion) from another biosynthetic route. The incorporation of the intact glucose molecule is more common among the actinomycetes than among the fungi.

(2) ***Secondary products related to nucleosides***:

The pentose phosphate pathway provides ribose (5 carbon) for nucleoside biosynthesis. Many secondary metabolites in this group are antibiotics and are produced mainly by actinomycetes and fungi. Examples are nucleoside antibiotics such as bleomycin.

(3) ***Secondary products derived through the Shikimate-Chorismate Pathway***:

Shikimic acid (C7) is formed by the condensation of erythrose-4-phosphate (C4) obtained from the PP pathway with phosphoenolypyruvate (C3) from the EMP pathway. It is converted to chorismic acid which is a key intermediate in the formation of numerous products including aromatic aminoacids, such as phynylalamine, tryrosine and tryptophan.

Chorismic acid is also a precursor for a number of secondary metabolites including chloramphenicol, p-amino benzoic acid, phenazines and pyocyanin which all have anticrobial properties.

The metabolic route leading to the formation of these compounds is therefore referred to as the shikimate pathway. In view of this central role of chorismic acid, however, the route is more widely known as the shikimate-chorismate route. The shikimate-chorismate route is an important route for the formation of aromatic secondary products in the bacteria and actinomycetes.

Examples of such secondary products include chloramphenicol and novobiocin. The route is less used in fungi, where the polyketide pathway is more common for the synthesis of aromatic secondary products.

(iv) ***The polyketide pathway***:

polyketide biosynthesis is highly characteristic of the fungi, where more secondary metabolites are produced by it than by any other. Indeed most of the known polyketide-derived natural products have been obtained from the fungi, a much smaller number being obtained from bacteria and higher plants.

The triose (C3) derived from glucose in the EMP pathway is converted via pyruvic acid to acetate, which occupies a central position in both primary and secondary synthesis. The addition of CO_2 to an acetate group gives a malonate group. The synthesis of polyketides is very similar to that of fatty acids. In the synthesis of both groups of compounds acetate reacts with malonate with the loss of CO_2.

By successive further linear reactions between the resulting compound and malonate, the chain of the final compound (fatty acid or polyketide) can be successively lengthened.

However, in the case of fatty acid the addition of each malonate molecule is followed by decarboxylation and reduction whereas in polyketides these latter reactions do occur.

Polyketides are classified as triketides, tetraketides, pentaketides, etc., depending on the number of ‘C2 units’. Thus, orsellenic acid which is derived from the straight chain compound with four ‘C2-units’ is a tetraketide. Although the polyketide route is not common in actinomycetes, a modified polyketide route is used in the synthesis of tetracyclines by Streptomyces griseus.

(5) ***Terpenes and steroids***:

The second important biosynthetic route from acetate is that leading via mevalonic acid to the terpenes and steroids. Microorganisms especially fungi and bacteria synthesize a large number of terpenes, steroids, carotenoids and other products following the 'isoprene rule'. The central point of this rule is that these compounds are all derivatives of isoprene, the five-carbon compound. Simply put the isoprene rules consist of the following:

(i) Synthesis of mevalonate from acetate or leucine

(ii) Dehydratopm and decarboxylation to give isoprene followed by condensation to give isoprenes of various lengths.

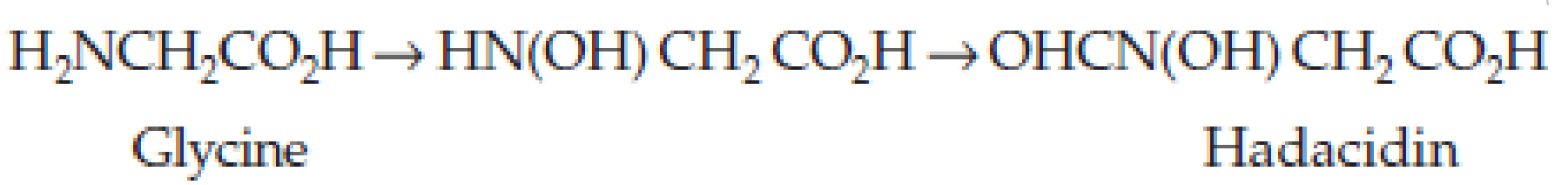
(iii) Cyclization (ring formation) e.g., to give steroids

(iv) Further modification of the cyclised structure. The route leads to the formation of essential steroid hormones of mammals and to a variety of secondary metabolites in fungi and plants. it is not used to any extent in the actinomycetes.

(6) ***Compounds derived from amino acids***:

The amino acids are derived from various products in the catabolism of glucose. Serine (C3N) and glycine (C2N) are derived from the triose (C3) formed glucose; valine (C5N) is derived from acetate (C3); aspartatic acid (C4N) is derived from oxeloacetic acid (C4) while glutamic acid(C5N) is derived from oxoglutamic acid (C5).

Secondary products may be formed from one, two or more amino acids. an example of the first group (with one amino acid group) is hadacidin which inhibits plant tumors and is produced from glycine and produced by Penicillium frequentants according to the formula shown below



Other examples are the insecticidal compound, ibotenic acid (Amanita factor C) produced by the mushroom *Amanita muscaria* and psilocybin, a drug which causes hallucinations and produced by the fungus *Psiolocybe*

Among the secondary products derived from two amino acids are gliotoxin which is produced by members of the Fungi Imperfecti, especially *Trichoderma* and which is a highly active anti-fungal and antibacterial and Arantoin, an antiviral drug also belongs to this group.

The secondary products derived from more than two amino acids include many which are of immense importance to man. These include many toxins from mushrooms e.g the *Aminita* toxins (phalloidin, amanitin) peptide antibiotics from *Bacillus* app and a host of other compounds.

An example of a secondary metabolite produced from three amino acids is malforming A which is formed by *Aspergillus spp*. It induces curvatures of beam shoots and maize seedlings. It is formed from L-leucine, D-leucine, and cysteine