NATIONAL OPEN UNIVERSITY OF NIGERIA

NSC 207

Medical Biochemistry

Module 1

NSC 207 (Medical Biochemistry II)
Module 1

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Unit 1 Glycolysis

1.0 Introduction

Glycolysis is the sequence of reactions that converts glucose into pyruvate with the production of ATP. In aerobic organisms, glycolysis is the prelude to the citric acid cycle and the electron transport chain, where most of the energy contained in glucose is released. Under aerobic conditions, pyruvate enters the mitochondria where it is completely oxidized to CO_2 and H_2O. If the supply of oxygen is insufficient, e.g. in actively contracting muscle, pyruvate is converted into Lactate. In some anaerobic organisms, pyruvate is transformed into ethanol. The formation of ethanol and Lactate from Glucose are examples of fermentations. Reactions of glycolysis take place in the cytosol. Glycolysis is sometimes called the Embden Meyerhof pathway, after Gustav Embden and Otto Meyerhof who made significant contributions to its elucidation in 1940.

2.0 Objectives

At the end of this unit, you should be able to:

- explain the concept of glycolysis
- state the importance of the glycolytic pathway
- enumerate the different reactions which make up the pathway and the enzymes which catalyze these reactions.

3.0 Main Content

3.1 The Glycolytic Pathway

The glycolytic pathway has a dual role (i) It degrades glucose to generate ATP and (ii) It provides building blocks for synthetic reactions. The rate of conversion of glucose into pyruvate is regulated to meet these 2 major cellular needs. Glycolysis occurs, with variation in nearly all organisms, indicating that it is one of the most ancient metabolic pathways.

3.2 The Stages of Glycolytic Pathway

The glycolytic pathway can be divided into three stages.

Stage One

The conversion of Glucose to fructose 1,6 Biphosphate. This stage comprises of 3 steps- a phosphorylation, an isomerization and another phosphorylation.

1. Glucose is phosphorylated by ATP to form glucose 6-phosphate. This reaction is catalyzed by Hexokinase (An enzyme that transfers a phosphoryl group from ATP to an acceptor is called a kinase).
2. Glucose + ATP $\longrightarrow$ Glucose 6-phosphate + ADP + Pi
3. Glucose 6-phosphate is isomerized to Fructose 6-phosphate. The reaction is catalyzed by Phospho glucose isomerase.
4. Fructose 6-phosphate is phosphorylated by ATP to Fructose 1,6-biphosphate. Fructose 6-phosphate + ATP $\longrightarrow$ Fructose 1,6-biphosphate +ADP + H+

This reaction is catalyzed by phosphofructokinase, an allosteric enzyme. The pace of glycolysis is critically dependent on the level of this enzyme. Its catalytic activity is controlled by ATP and other metabolites.

Stage Two

This stage of glycolysis consists of 4 steps, starting with the splitting of Fructose 1,6 biphosphate to yield glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The remaining steps in glycolysis involve 3 carbon units rather than 6 carbon units.

4 Fructose 1,6-biphosphate $\longrightarrow$ Dihydroxyacetone phosphate + Glyceraldehyde 3-phosphate

The reaction is catalyzed by aldolase. The 2 products formed are isomers. Dihydroxyacetone phosphate is a ketose while glyceraldehydes 3-phosphate is an aldose. The reaction proceeds readily from DAP to TPI through the action of the enzyme triose phosphate isomerase. Thus, 2 molecules of glyceraldehyde 3- phosphate are formed from one molecule of Fructose 1,6 biphosphate.

5 Glyceraldehyde 3-phosphate $\longrightarrow$ Dihydroxyacetone phosphate.

6 Conversion of glyceraldehydes 3-phosphate to 1,3 –diphosphoglycerate, catalysed by glyceraldehyde 3-phosphate dehydrogenaseGly 3-P + NAD+ + Pi $\longrightarrow$ 1,3 DPG + NADH + H+

7 1,3 Diphosphoglycerate is converted to 3-phosphoglycerate, and ATP is generated.

The rxn is catalyzed by phosphoglycerate kinase.

1,3 Diphosphoglycerate + ADP $\longrightarrow$ 3-Phosphoglycerate + ATP

Stage Three

In this stage, three steps are involved leading to the generation of pyruvate

8 3-phosphoglycerate is converted to 2-phosphoglycerate, through the action of 2-phosphoglyceromutase.

9 2-phosphoglycerate is converted to phosphoenolpyruvate by Enolase

2-Phosphoglycerate $\longrightarrow$ Phosphoenol pyruvate + H_2O

9 PEP is converted to Pyruvate with the generation of ATP, the rxn being catalyzed by pyruvate kinase.

PEP + ADP + Pi $\longrightarrow$ Pyruvate + ATP

The net reaction in the conversion of Glucose into pyruvate is

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

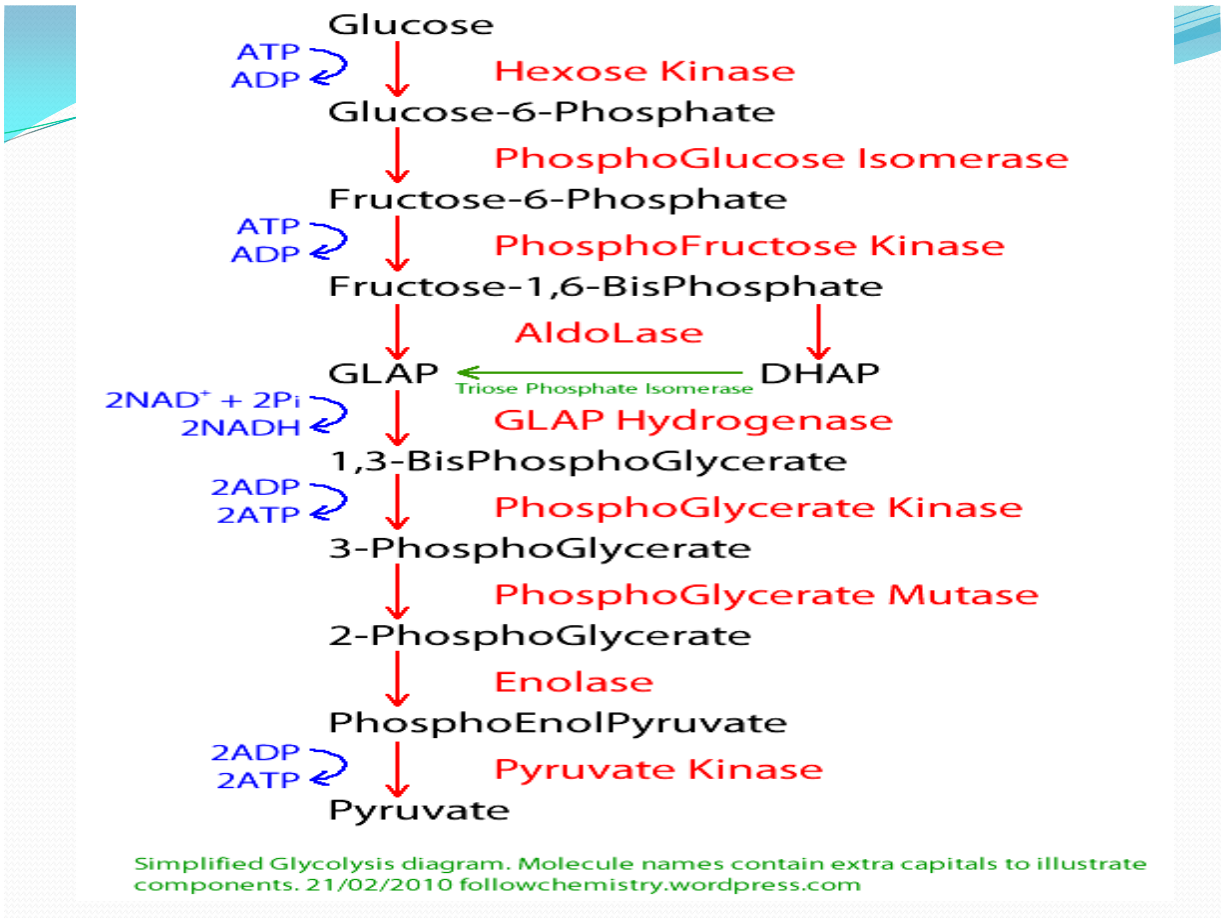

Fig 1.1: The Glycolytic Pathway

4.0 Conclusion

5.0 Summary

In this unit, you have been exposed to:

- The concept of Glycolysis
- The stages of glycolysis reactions.

6.0 Self Assessment Exercise

Activity: As directed in the Laboratory.

1. Explain the concept of Glycolysis
2. State the importance of the glycolytic pathway
3. Enumerate the different reactions which make up the pathway and the enzymes which catalyze these reactions.

7.0 References/Further Reading

Katherine, M. A. Rogers & William N. Scott. (2011). Nurses! Test yourself in anatomy and physiology

Kathryn, A. Booth, Terri. D. Wyman (2008). Anatomy, physiology, and pathophysiology for allied health

Keith L.M, Persuade T.V.N (2006). The Developing Human Clinically Oriented Embryology 8th Edition Lippincott Williams & Wilkins

Kent, M. Van De Graff, R.Ward Rhees, Sidney P. (2010). Schaum's outline of human anatomy and physiology (3rd ed.).

Philip, T. (2012). Seeley's principles of anatomy & physiology. (2nd ed.).

Sadler, T.W (2004). Langman's Medical Embryology. (9th ed.).

Unit 2 Glycolysis 2

1.0 Introduction

We continue our discussion of the glycolytic pathway. 2 ATP molecules are produced in the course of the pathway. However, more ATP is produced when pyruvate is completely oxidized to CO_2 and H_2O in the mitochondria. The glycolytic pathway is regulated through the activities of 3 enzymes that catalyze its irreversible reactions. However, the most important control element of glycolysis is the enzyme phosphofrctokinase (PFK), the enzyme catalyzing the first irreversible step unique to the pathway. Pyruvate has 3 fates- It may be converted to acetyl coA, ethanol or Lactate. Clinical conditions associated with impaired glycolysis include Lactic acidosis and Pyruvate kinase deficiency.

2.0 Objectives

At the end of this unit, you should be able to:

- give the gross and net ATP yield of glycolysis
- list the glycolytic regulatory enzymes and their corresponding effectors.
- explain the fates of Pyruvate
- list and explain some disorders of Glycolytic pathway.

3.0 Main Content

3.1 Consumption and Generation of ATP in Glycolysis

The following table provides an outline of how ATP is consumed and produced during glycolysis, with a net production of 2 ATP molecules. Most of the energy contained in glycolysis is harvested in the TCA cycle and the electron transport chain.

Reaction		ATP change per glucose
Glucose	⟶	Glucose 6-Phosphate -1
Fructose6-phosphate	⟶	fructose 1,6-biphosphate -1
2 1,3-Biphosphoglycerate	⟶	2 3-phosphoglycerate +2
2 PEP	⟶	2 Pyruvate +2
		Net= +2

3.2 Regulation of Glycolysis

The glycolytic pathway has a dual role (i) It degrades glucose to generate ATP and (ii) It provides building blocks for synthetic reactions. The rate of conversion of glucose into pyruvate is regulated to meet these 2 major cellular needs. Enzymes catalyzing essentially irreversible reactions are potential sites of control. In glycolysis, the reactions catalyzed by Hexokinase (HK), phosphofructokinase (PFK) and Pyruvate kinase (PK) are virtually irreversible, and so these enzymes play regulatory as well as catalytic roles.

However, PFK is the most important control element in glycolysis. The enzyme is inhibited by:

High levels of ATP. ATP binds to a highly specific regulatory site that is distinct from the catalytic site. The inhibitory action is reversed by AMP. The activity of the enzyme increases when the ATP/AMP ratio is lowered.

High levels of Citrate, which indicates that biosynthetic precursors are abundant.

Citrate inhibits PFK by enhancing the inhibitory effect of ATP. Hexokinase and Pyruvate kinase also participate in regulating the rate of glycolysis. In general, the enzyme catalyzing the committed step (the first irreversible reaction unique to a pathway) in a metabolic sequence is the most important control element in the pathway. PFK is most active when the cell needs both energy and building blocks. It is moderately active when either energy or a carbon skeleton is needed. The enzyme is almost switched off when both are abundant.

Hexokinase and Pyruvate kinase also participate in regulating the rate of glycolysis. Pyruvate kinase from muscle and liver is allosterically inhibited by ATP, so the conversion of PEP to pyruvate is blocked when the energy charge is high. Hexokinase is allosterically inhibited by glucose 6 –phosphate. The level of F6P increases when PFK is blocked, and so there is a corresponding increase in the level of G6P, which is in equilibrium with F6P.Hence, inhibition of PFK leads to the inhibition of HK.

3.3 Regulation of Glycolysis

The fate of pyruvate in the generation of metabolic energy in different organisms and different kinds of cells varies.

1. Pyruvate can be converted to Ethanol, Lactate or Acetyl CoA.

Ethanol is formed from pyruvate in yeast and several other microorganisms in 2 steps as follows

a. Pyruvate + H^+ $\longrightarrow$ Acetaldehyde + CO2
b. Acetaldehyde + NADH + H+ $\longrightarrow$ Ethanol + NAD+

The conversion of glucose into ethanol is called alcoholic fermentation. The net reaction is

$$\text{Glucose} + 2P_i + 2ADP + 2H^+ \longrightarrow 2\text{Ethanol} + 2CO_2 + 2ATP + 2H_2O$$

2. Lactate is formed from pyruvate in many microorganisms as well as in cells of higher organisms when the amount of oxygen is limiting e.g. in muscle during intense activity.

Pyruvate + NADH + H+ $\longrightarrow$ L-Lactate + NAD+

The net reaction for the conversion of glucose to lactate is

Glucose + 2Pi + 2ADP $\longrightarrow$ 2Lactate + 2ATP + $2H_2O$

3. A lot of energy is derived aerobically by means of TCA cycle and electron transport chain. The entry point to this oxidative pathway is acetyl coenzyme A (Acetyl CoA), which is formed inside mitochondria by the oxidative decarboxylation of Pyruvate:

Pyruvate + NAD^+ + CoA $\longrightarrow$ AcetylcoA + CO_2 + NADH

The reaction is catalyzed by the Pyruvate dehydrogenase complex

3.4 Clinical Conditions Associated with impaired Glycolysis

Lactic Acidosis
This is the most frequent form of metabolic acidosis. It can occur as a result of overproduction of lactate, underutilization of Lactate or inhibition of pyruvate dehydrogenase. It may also be as a result of rare congenital disorders where the mitochondria do not function at full capacity or diabetic ketoacidosis as well as liver/kidney disease. It is characterized by Lactate levels> 5mM/L and serum pH<7.35

Symptoms: Nausea, Vomiting, Hyperventilation, Irregular heart rate.

Pyruvate Kinase Deficiency
A rare genetic defect of glycolysis causes haemolytic anaemia. Glycolytic intermediates close to the pyruvate kinase step accumulate, whereas pyruvate and Lactate concentrations decrease. Lysis of the RBCs may cause jaundice from increased Bilirubin.

4.0 Conclusion

5.0 Summary

In this unit, you have learnt about the following:

- Consumption and generation of ATP in Glycolysis
- Regulation of Glycolysis
- Regulation of Glycolysis
- Clinical conditions associated with impaired Glycolysis.

6.0 Self Assessment Exercise

Activity: Your course facilitator would contact you on the expected activity you are to perform.

1. Give the gross and net ATP yield of glycolysis

2. List the glycolytic regulatory enzymes and their corresponding effectors.
3. Explain the fates of Pyruvate
4. List and explain some disorders of Glycolytic pathway

7.0 References/Further Reading

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Keith L.M, Persuade T.V.N (2006). The Developing Human Clinically Oriented Embryology 8th Edition Lippincott Williams & Wilkins

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Philip, T. (2012). Seeley's principles of anatomy & physiology 2nd edition.

Sadler, T.W (2004). Langman's Medical Embryology 9th edition.

Unit 3 Tricarboxylic Acid Cycle

1.0 Introduction

The citric acid cycle was discovered by Hans krebs in 1937 and received Nobel prize for the discovery in 1953. The cycle was therefore named after him as kreb's cycle. The cycle is also known as citric acid cycle or tricarboxylic acid cycle. The citric acid cycle is the final common pathway for the oxidation of fuel molecules (protein, fatty acids and carbohydrates) to energy, carbon dioxide and water. Without this cycle, most of the food we eat cannot be converted to energy. Most of these fuel molecules are metabolized to acetyl Coenzyme A (acetyl CoA) or intermediates of the cycle. The acetyl CoA generated is then fed into the cycle and condenses with oxaloacetate. Two molecules of CO_2 is liberated, the energy released is conserved in the reduced electron carriers NADH and $FADH_2$. In the final stage the conserved energy is released and stored as ATP.

2.0 Objectives

At the end of this unit, you should be able to:

- describe TCA cycle in detail
- explain the Amphibolic nature of the TCA cycle
- explain the Anaplerotic nature of the TCA cycle
- describe the relationship between this cycle and Beriberi (a neurological disease)
- give the summary of oxidative phosphorylation
- give examples and describe the inhibition of electron transport chain.

3.0 Main Content

3.1 Description of TCA Cycle

In eukaryotes, TCA cycle takes place in the mitochondria because all the enzymes of the cycle are located inside the mitochondria matrix. The TCA cycle is an important source of precursors or building blocks for the synthesis of molecules such as amino acids, purine bases, cholesterol and porphyrins.

The cycle starts when a four-carbon compound (oxaloacetate) condenses with a two carbon acetyl unit of acetyl coA to yield a six carbon tricarboxylic acetate (citrate). In a cyclic series of reactions (figure 2.1) the isomer of citrate (isocitrate) is oxidatively decarboxylated (one molecule of CO_2 is released). The resulting five carbon compound, α-ketoglutarate is also oxidatively decarboxylated (another molecule of CO_2 is also released) to yield a four-carbon compound (succinate).

Oxaloacetate, the starting material is eventually regenerated through the formation of fumarate and malate. Oxaloacetate's function in kreb's cycle can be described as catalytic in nature because the compound participates in the oxidation reaction and it is regenerated at

the end of the cycle. Carboxylation of pyruvate is the major source of oxaloacetate as the starting materials for the cycle.

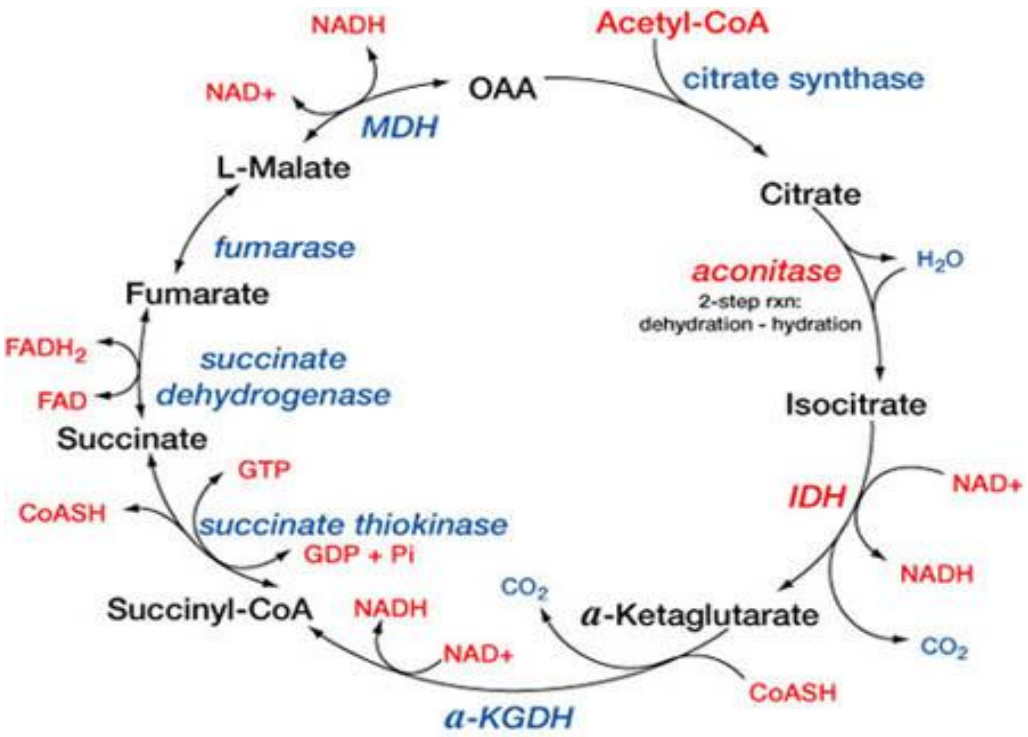

Fig. 3.1: The TCA Cycle showing the Enzymes and the Intermediates

Source: google images

3.2 The Amphibolic Nature of the TCA Cycle

The citric acid cycle functions as catabolic pathway when it is used to break down acetyl CoA to two molecules of CO_2, water and energy. Whenever the energy level of the cell is low, catabolic pathway is favoured. The sole purpose of the TCA cycle when it is operating as catabolic pathway is the oxidation of acetate to CO2, with concomitant conservation of the energy of oxidation as reduced coenzymes and eventually as ATP.

The cycle also functions as anabolic pathway when there is sufficient energy reserve in the cell. The pathway is used to supply building blocks for the synthesis of various biological molecules. The function of the cycle at a particular time is determined by the energy conditions of the cell, because of its dual functions, it is referred to as an *amphibolic* pathway.

3.3 The Anaplerotic Nature of the TCA Cycle

Basically, the TCA cycle has a single substrate, and the substrate is acetylco A. In most cells, however, there is considerable withdrawal and addition of intermediates into and out of the cycle, which occurs in addition to the primary function of the cycle. Such side reactions

serve two main purposes: one, to provide for the synthesis of compounds derived from any of several intermediates of the cycle and to replenish the supply of intermediates in the cycle as needed to prevent the shutting down of the cycle

Oxaloacetate and α-ketoglutarate are used in the synthesis of several amino acids. Citrate is the source of the acetyl coA in the cytosol, which is used for the synthesis of fats, other lipids and some amino acids. These are some of the major drains on the TCA cycle.

Reactions that replenish the intermediates in the TCA cycle are termed *Anaplerotic*. One of such reactions is the conversion of pyruvate to oxaloacetate. It is not necessary to replenish the intermediate that is used in a biosynthetic pathway directly, as the replenishment of any intermediate will occur by a feeding-in process at any point in the cycle. For example, when carbohydrates are being metabolized, the TCA cycle intermediates are replenished by production of oxaloacetate from pyruvate.

3.4 The Relationship between TCA Cycle and Beriberi

Beriberi, a neurological and cardiovascular disorder is caused by a dietary deficiency of thiamin also called vitamin B1. Beriberi is also occasionally seen in alcoholics who are severely malnurished and thus thiamine deficient.

The disease is characterized by neurologic and cardiac symptoms such as pain in the limbs and distorted skin sensation. The heart may be enlarged and may eventually lead to paralysis. Which biochemical processes might be affected by a deficiency of thiamine?

Thiamine pyrophosphate is the prosthetic group of two important TCA cycle enzymes; pyruvate dehydrogenase and α-ketoglutatrate dehydrogenase. In beriberi, the levels of pyruvate and α-ketoglutarate in the blood are higher than normal.

The reason why vitamin B1 deficiency leads to neurological disorders is because the nervous system relies essentially on glucose as its only fuel. In contrast, most other tissues can use fat as a source of fuel (Acetyl CoA) for the citric acid cycle. The pyruvate dehydrogenase complex is required to convert pyruvate (the end product of glycolysis) to Acetyl CoA. When the enzymes are inactivated due to thiamine deficiency, energy production in the nervous system is shut down. The consequence of this are the symptoms of beriberi listed above.

3.5 Summary of Oxidative Phosphorylation

The reduced coenzymes (NADH is called the reduced form of nicotinamide adenine dinucleotide and $FADH_2$ is called the reduced form of flavine adenine dinucleotide) derived from the TCA cycle are themselves oxidized when they released their protons and electrons. The electrons are transferred to oxygen, the final electron acceptor through a complex chain of electron-carrying molecules known as the electron transport chain. During the electron transferring process, large amount of energy is released and it is conserved in the form of ATP. This process is called oxidative phosphorylation.

3.6 Inhibitors of Electron Transport Chain

Inhibitors of electron transport chain were found to be useful asbarbiturate drugs, antibiotics and insecticides especially those that are selective. Examples include: Amytal (a barbiturate drug), rotenone (a plant product commonly used as an insecticide) and piericidin A and oligomycin (antibiotics) block the electron flow through the respiratory chain and thereby shut down energy production in their respective targets.

4.0 Conclusion

5.0 Summary

In this unit, you have learnt about the following:

- Description of TCA cycle
- Amphibolic Nature of the TCA Cycle
- Anaplerotic Nature of the TCA Cycle
- Relationship between TCA cycle and Beriberi
- Summary of oxidative phosphorylation
- Inhibitors of electron transport chain.

6.0 Self Assessment Exercise

Activity: Your course facilitator would inform you about a practical assignment you expected to carry out.

1. Describe TCA cycle in detail
2. Explain the Amphibolic nature of the TCA cycle
3. Explain the Anaplerotic nature of the TCA cycle
4. Describe the relationship between this cycle and Beriberi (a neurological disease)
5. Give the summary of oxidative phosphorylation
6. Give examples and describe the inhibition of electron transport chain

7.0 References/Further Reading

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