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Subject	Final Exam - Chapter Nineteen

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ساعات الدوام الرسمي

السبت - الخميس 12:30ظهراً -11:00 ليلاً
الجـمعة 2:00 ظهراً -11:00 ليلاً

ALQUSOUR ACADEMY
خاص
للفصل الدراسي الصيفي
2012 - 2013

Prod. Date
04/08/2013

Pages
14

Price
40

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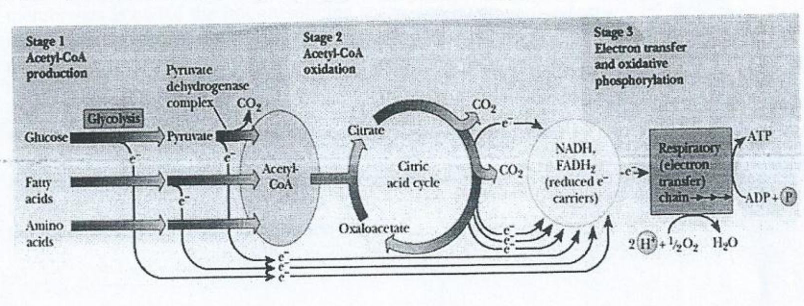
CITRIC ACID CYCLE

Introduction:

- The citric acid cycle is considered the central metabolic pathways in the cell; it occurs in the mitochondria. Nutrients enter the citric acid cycle by forming the high energy complex Acetyl-Co-A.

- The cycle starts by joining acetyl-Co-A to **Oxaloacetate** to form citric acid and ends by releasing 2 molecules of CO_2 and the original molecule oxaloacetate.



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- Almost any type of nutrients can enter the cycle in the form of acetyl-Co-A or any other intermediate products, the reverse is also true, and any nutrient can be extracted from the cycle by building up the nutrient from intermediate molecules of the cycle.

- So pyruvate from glucose metabolism, amino acids from protein metabolism and fatty acids from lipids all can enter the cycle to be broken down to CO_2 or can be built from the cycle.

- The citric acid cycle is also called Krebs cycle, and Tricarboxylic acid cycle.

☒ **<u>Summary of citric acid cycle</u>**

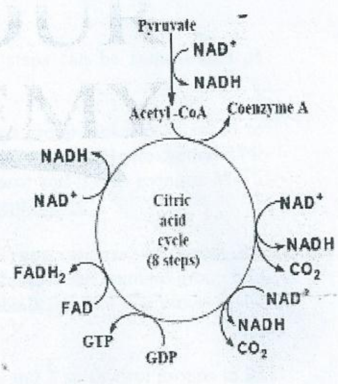
- The citric acid cycle is an **Amphibolic** cycle which means that it participates in both, catabolism and anabolism.

- In catabolism (oxidative breakdown of nutrient) large molecules can enter the cycle either in the form of acetyl-Co-A or in the form of cycle intermediates, to be broken down finally to CO_2.

- On the other hand, anabolism (reductive synthesis of biomolecules) occurs when the cell needs to build up large molecules from smaller ones, intermediates of the cycle can be used to build up large molecules. That's what we mean by amphibolic.

- Most catabolic reactions are oxidation reactions, in which electrons are lost, as we said earlier the ultimate electron acceptor is oxygen with water as the product. In the citric acid cycle electrons are lost to coenzymes; the transport of electrons from the coenzymes to oxygen is the function of another pathway namely the electron transport chain (**ETC**) and oxidative phosphorylation.

- The citric acid cycle takes place in **mitochondria.** The mitochondrion has an inner and an outer membrane, the region inside the inner membrane is called the mitochondrial matrix, and the region between the 2 membranes is called the intermembrane space, the inner membrane is tight barrier, so compounds need specific membrane proteins to cross. Where does glycolysis take place?



- The reactions of citric acid cycle take place in the matrix, **except** the reaction in which FAD is the intermediate electron acceptor, because the enzyme that catalyzes the FAD-linked reaction is an integral part of the inner membrane and linked directly to ETC.

- The citric acid cycle consists of 8 **steps, 4** of them (steps, 3, 4, 6, 8) are **oxidation reactions.** The oxidizing coenzyme is **NAD**$^+$ in all of these steps except step 6 in which **FAD** is the oxidizing agent.

- The citric acid cycle produces only one high energy molecule; this is **GTP** (Guanosine Triphosphate) which can then be used to generate an ATP molecule.

☒ **The conversion of pyruvate to Acetyl Co-A**

- Pyruvate (3-carbon molecule) is converted to Acetyl-CoA (acetyl has 2 carbon atoms) and 1 CO2 molecule, this is done by the enzyme complex **Pyruvate Dehydrogenase Complex (PDH)** (complex means it is composed of more than 1 enzyme).

- These enzymes are ***Pyruvate Dehydrogenase, Dihydrolipoyl Transacetylase, Dihydrolipoyl Dehydrogenase, Pyruvate Dehydrogenase Kinase*** and ***Pyruvate Dehydrogenase Phosphatase.***

- The first 3 enzymes of the complex are the ones involved in the reactions to convert pyruvate to acetyl-CoA, whereas the last 2 are important to control the function of the PDH complex and they are found on a single polypeptide.

- Although we consider the reaction to convert pyruvate to acetyl-CoA as a single reaction by the following equation:

$$\text{Pyruvate} + \text{CoA-SH} + \text{NAD}^+ \xrightarrow{\textbf{PDH}\text{ Complex}} \text{Acetyl-CoA} + \text{Co2} + \text{NADH} + \mathbf{H}^+$$

- It is an exergonic reaction (dela G0'= -33.4 kJ mol-1) and the NADH can then be used to generate ATP via ETC.

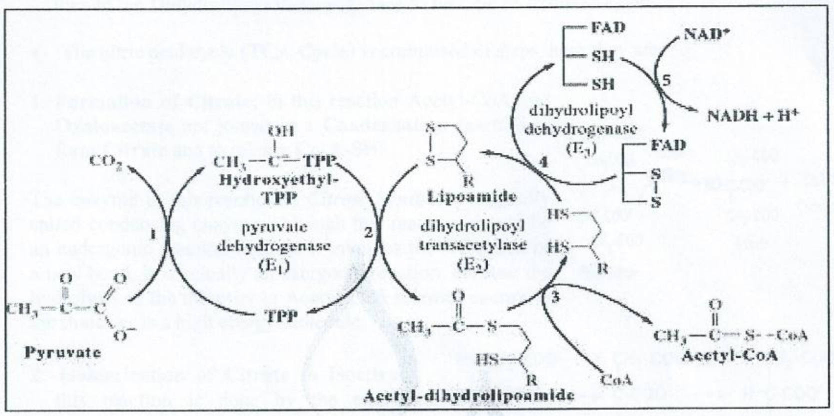
- The actual reaction takes place in *5* steps, and these steps can be summarized as follows:

1. Pyruvate loses **CO2** and is converted to a Hydroxyethyl group which is attached to Thiamine Pyrophosphate **(TPP)** which acts as a coenzyme, to form Hydroxyethyl-TPP **(HETPP),** this step is catalyzed by ***Pyruvate Dehydrogenase*** and it also requires **Mg**$^{+2}$. The coenzyme TPP is attached to the enzyme by non-covalent bonds.

2. Step 2 is catalyzed by the enzyme ***Dihydrolipoyl Transacetylase,*** to which the coenzyme **Lipoic Acid** is covalently attached by an amide bond to the ε-amino group of a lysine side chain. **Lipoic acid** is a vitamin rather than a metabolite of a vitamin as is the case with many other coenzymes.

This coenzyme has a **disulfide group** in its oxidized form and 2 sulfhydryl groups in its reduced form. In this step the hydroxyethyl group is oxidized to an acetyl **group** and is attached to the lipoic acid, to form a thioester which is a high energy molecule.

3. Step 3 is also done by the enzyme ***Dihydrolipoyl Transacetylase,*** in this step the acetyl group is transferred from lipoic acid to **CoA** to form Acetyl-CoA, and the lipoic acid is in its reduced form.



By now pyruvate has lost CO_2 and an acetyl group has been attached to CoA to form acetyl-CoA, but the lipoic acid is in its reduced form and has to be converted to its oxidized form to be used for further reactions.

Step **4** and step **5**, are done by the enzyme ***Dihydrolipoyl Dehydrogenase,*** which has the coenzyme **FAD** attached to it by <u>non-covalent bonds</u>, this enzyme reoxidizes the reduced lipoic acid from the sulfhydryl to the disulfide form, as a result FAD is reduced to FADH2 which is then reoxidized to convert the oxidizing agent NAD^+ to NADH.

- The PDH complex is composed as we said from five enzymes, in reality, the enzyme complex has a very complicated structure. The complex is formed of ***24 Dihydrolipoyl Transacetylase*** molecules every 3 of them **(trimer)** are present at a corner of an imaginary cube.
- ***Pyruvate Dehydrogenase*** is a dimer (each consists of α, β subunits), there are **12 αβ dimers** which occupy the edges of the cube. ***Dihydrolipoyl Dehydrogenase*** is also a **dimer,** there are 6 dimers of it each one occupies a face of the cube.
- This compact structure has 2 great advantages over an arrangement in which the various components are widely dispersed:

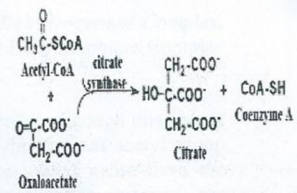
1. The various steps of reaction can take place more efficiently because the enzymes and reactants are closer to each other.

2. The regulatory control can be applied more efficiently.

- Transacetylase molecules are in a central position of the complex. The lipoic acid and the lysine side chain to which it is bounded are long enough to act as a swinging arm that moves from the pyruvate dehydrogenase enzyme to get the acetyl group and then to the Dihydrolipoyl dehydrogenase to become re-oxidized again.

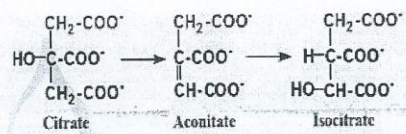
- The citric acid cycle **(TCA Cycle)** is composed of steps, here they are:

1. **Formation of Citrate;** in this reaction Acetyl-CoA and **Oxaloacetate** are joined in a **Condensation** reaction to form **Citrate** and to release **Co-A-SH.**



The enzyme in this reaction is ***Citrate Synthase,*** originally called condensing enzyme, although this reaction should be an endergonic reaction because it involves the formation of a new bond, it is actually an exergonic reaction, because the hydrolysis of the thioester in Acetyl-CoA releases energy as the thioester is a high energy molecule.

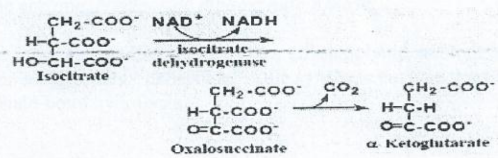
2. **Isomerization of Citrate to Isocitrate,** this reaction is done by the enzyme **Aconitase.** This reaction requires Fe^{+2} as a cofactor.



In here we can see that Citrate (an achiral compound) is converted to isocitrate which is chiral, this is done because the enzyme binds citrate in an unsymmetrical binding site. After that, citrate is converted to **cis-aconitate** which is an intermediate molecule in this reaction, which is then converted to isocitrate.

3. **Oxidative decarboxylation**: in here, the first oxidation reaction of the pathway occurs, in which α-Ketoglutarate is formed and CO_2 is released, the enzyme that catalyzes this reaction is called ***Isocitrate Dehydrogenase.***

This reaction happens actually in 2 steps the first one is that **Isocitrate** is oxidized to **Oxalosuccinate,** which then remains bound to the enzyme, in the next step **Oxalosuccinate** is decarboxylated and the CO_2 and **α-Ketoglutarate** are released. Because in this step there is oxidation, there must be a reduction reaction, so NAD^+ is reduced to NADH in this reaction.

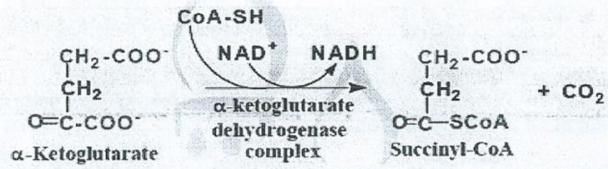


NOTE: through oxidative phosphorylation, each NADH molecule produced **2.5** ATP molecules].

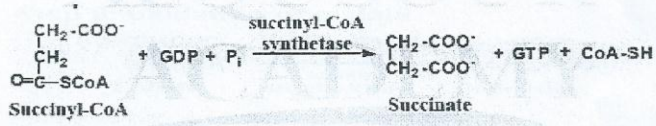
4. This reaction is also an **oxidative decarboxylation reaction (2nd oxidation),** in here, α-Ketoglutarate is converted to **Succinyl-CoA** by the addition of CoA and CO_2 is released, again since this is an oxidation reaction another molecule has to be reduced, this is again NAD^+ which is converted to NADH.

The enzyme that catalyzes this reaction is ***a-Ketoglutarate Dehydrogenase Complex,*** this enzyme works in a similar way to the enzyme Pyruvate Dehydrogenase Complex and also requires **TPP, FAD, lipoic acid** and $\mathbf{Mg^{+2}}$.

NOTE: (by this point the reaction has released 2 CO_2 molecules, although this should mean that these 2 carbon atoms have been released from the original acetyl group attached to acetyl-CoA, this is not the case; the 2 carbon atoms came from the oxaloacetate, and the 2 carbon atoms from the acetyl-CoA are used to regenerate the oxaloacetate.



5. **Formation of Succinate,** in this reaction Succinyl-CoA is hydrolyzed to produce **Succinate** and **CoA-SH,** this reaction is done by the enzyme ***Succinyl-CoA synthetase,*** and the reaction is coupled to the production of **GTP** from **GDP.**



NOTE: [The phosphate group is then transferred from the **GTP** to an **ADP** molecule to produce ATP by the enzyme, ***Nucleoside diphosphate Kinase.***

NOTE: synthetase is an enzyme that creates a new covalent bond and requires direct input of energy from a high energy phosphate, while **synthase** enzyme does not require energy from phosphate-bond hydrolysis.

6. **Formation of Fumarate (FAD-linked oxidation)**: in this step **Succinate** is oxidized to form **Fumarate.** The enzyme that catalyzes this reaction is ***Succinate Dehydrogenase*** (an integral protein of inner membrane), since this is an oxidation reaction another substance has to be reduced, in this case it is **FAD not NAD^+**,which is then converted to FADH2.

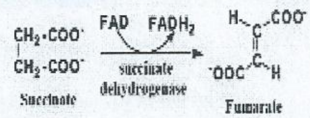
NOTE: [Because this enzyme contains FAD which is covalently attached **to the** enzyme it is sometimes called **a flavoprotein.** Because it also contains **iron** it is sometimes called Nonheme Iron Protein or Iron Sulfur protein.

NOTE: Each FADH2 when oxidized by the electron transport chain results in the formation of 1.5 ATP molecules rather than 2.5 ATP as in NADH]

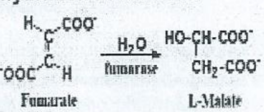
7. **Formation of L-Malate**, this is a hydration reaction in which water is added to **Fumarate** to produce **L-Malate,** the enzyme is ***Fumarase,*** and although malate has 2 enantiomers only L-Malate is produced.

8. **Regeneration of Oxaloacetate (final oxidation step)**: in this step, **Malate** is oxidized to **Oxaloacetate,** the original molecule produced by the reaction; the enzyme here is ***Malate Dehydrogenase.*** And another NAD^+ is reduced to NADH.**Oxaloacetate** then can react with another new **Acetyl-CoA** to start another cycle.

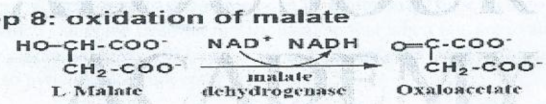
- **Step 6: oxidation of succinate to fumarate**



- **Step 7: hydration of fumarate**



- **Step 8: oxidation of malate**



☒ **Energy yield of citric acid cycle**

The overall citric acid cycle is an exergonic pathway. To calculate the number of ATP molecules that can be produced you have to remember that each NADH molecule when oxidized by the electron transport chain produces 2.5 ATP molecules, and that each FADH2 when oxidized produces *1.5* ATP molecules.

1. In the citric acid cycle 3 NADH molecules and 1 FADH2 molecule are produced so *(2.5*3+1.5)* = **9** ATP

2. The conversion of pyruvate to acetyl-CoA produced 1 NADH molecule, so 2.5 ATP molecules.

3. From the citric acid cycle 1 GTP molecule which is then converted to 1 ATP.

4. Overall, 1 + **9** + 2.5 = 12.5, but because each glucose molecule produces 2 pyruvate then 12.5 x 2 =25 ATP molecules.

5. From the glycolytic pathway, 2 NADH were produced, then 2 *x* 2.5 = *5* ATP molecules.

6. From the glycolytic pathway, 2 ATP were produced, then 5 +2 = 7 ATP molecules from glycolysis.

7. The total would be 25 ATP from the citric acid cycle and 7 from glycolysis then: *7+25* = 32 ATP molecules.

Note: Of individual reactions of the cycle, only one is strongly endergonic which is the oxidation of malate to oxaloacetate (delta G0'= +29.2 kJ mol-1).

⊠ Control of the Citric Acid Cycle:

- **Pyruvate Dehydrogenase enzyme** is inhibited by high levels of **ATP, acetyl Co-A** and **NADH**. and is activated by the decrease in their levels, this occurs through, the 2 regulatory enzymes of the Pyruvate Dehydrogenase Complex, these are, Pyruvate Dehydrogenase Kinase, Pyruvate Dehydrogenase phosphotase.

- When ATP levels are high, Pyruvate Dehydrogenase Kinase phosphorylates the complex leaving it inactive. While when the need arises for pyruvate dehydrogenase to be active, the hydrolysis of phosphate ester linkage (dephosphorylation) is catalyzed by Pyruvate Dehydrogenase Phosphatase which is activated by Ca^{+2}.

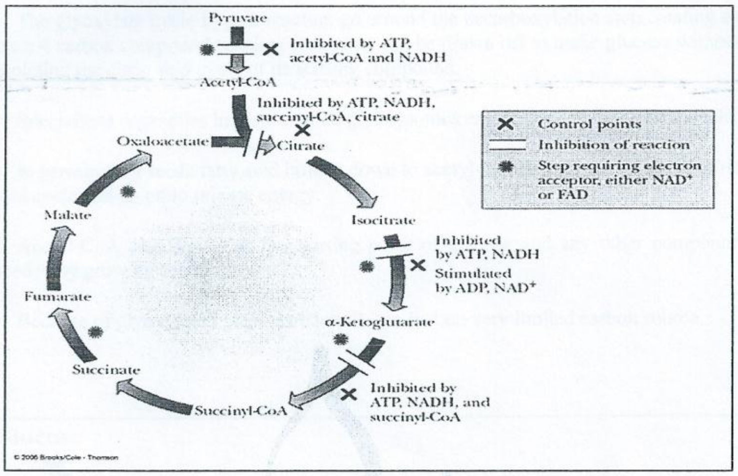
NOTE: The pyruvate dehydrogenase complex can be inhibited by high levels of acetyl-CoA which can come from fat metabolism, so that when fats are available for energy extraction there will be no need to send carbohydrates to the citric acid cycle, so pyruvate does not enter the cycle.

- **The control of the citric acid cycle occurs at 3 points:**

  1. **Citrate Synthase** which is inhibited by high levels of **ATP, NADH, Succinyl-CoA** and **Citrate**.

  2. **Isocitrate Dehydrogenase** is inhibited by **ATP** and **NADH,** and stimulated by **ADP** and NAD^+.

  3. **α-Ketoglutarate Dehydrogenase** is inhibited by high levels of **ATP, NADH** and **Succinyl-CoA.**



☒ What is the glyoxylate cycle?

• Plant and some bacteria, but not animals, can convert fat to carbohydrate through glyoxylate cycle. Animals can convert carbohydrate to fats, but not fats to carbohydrates

• Two enzymes are responsible for ability of plant and bacteria to produce glucose from fatty acid:

1- Isocitrate lyase cleaves isocitrate to produce succinate and glyoxylate:

isocitrate→ succinate + glyoxylate

2- Malate synthase catalyze the reaction of glyoxylate with acetyl-CoA to produce malate:

glyoxylate + acetyl-CoA → malate

• Then malate converts to oxaloacetate by *malate dehydrogenase* enzyme after that oxaloacetate converted to glucose (gluconeogensis).

• Because animal lack the 2 enzymes which involve in glyoxylate cycle it can't convert fatty acid to glucose.

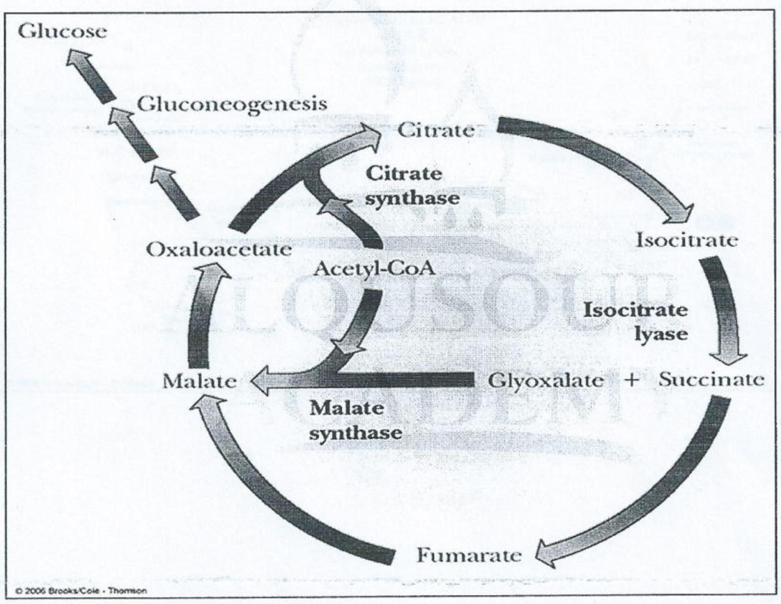
- The glyoxylate cycle bypass reaction go around the decarboxylation step creating an extra 4 carbon compound (oxaloacetate) that can be drawn off to make glucose without depleting the citric acid cycle of its starting compound.

- Specialized organelles in plants called glyoxysomes are the sites of glyoxylate cycle.

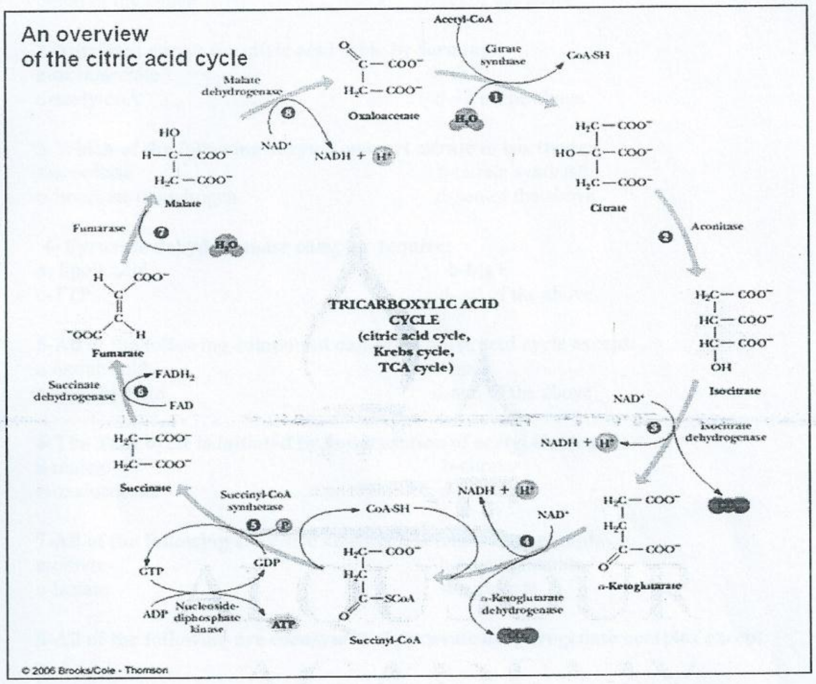
- In germinating seeds fatty acid broken down to acetyl-CoA which can enter the citric acid cycle and go on to release energy.

- Acetyl-CoA also serves as the starting point of glucose and any other compound needed by growing seedling.

- Because of glyoxylated pathway bacteria can live on very limited carbon source.



Overview of the citric acid cycle:



Questions:

1-The citric acid cycle is an amphibolic cycle means it participates in:
a-anabolism
b-catabolism
c-non of the above
d-all of the above

2-Nutrieant enters the citric acid cycle by forming:
a-oxaloacetate
b-CO2
c-acety-coA
d-all of the above

3-Which of the following enzyme convert citrate to isocitrate:
a-aconitase
b-citrate synthase
c-isocitrate dehydrogen
d-non of the above

4- Pyruvate dehydrogenase complex require:
a- lipoic acid
b-Mg+
c-TTP
d- all of the above

5-All of the following compound can enter citric acid cycle except:
a-amino acid
b-lipid
c-carbohydrate
d-non of the above

6-The TCA cycle is initiated by condensation of acetyl Co-A and:
a-malate
b-citrate
c-oxaloacetate
d-pyruvate

7-All of the following are citric acid cycle intermediate except:
a-citrate
b-a-ketoglutarate
c-lactate
d-succinate

8-All of the following are coenzymes of pyruvate dehydrogenase complex except:

a. CoA
b. FAD
c. NAD+
d. Lipoic acid
e. ATP

9-When muscle tissue is exercising under anaerobic conditions, the production of --------------is important it assures a continuous supply of NAD+.

a. Glycogen
b. Lactate
c. Fructose
d. Glucose-6-phosphate
e. Pyruvate

Answer sheet

Question#	Answer
1-	d-all of the above
2-	c-acety-coA
3-	a-aconitase
4-	d- all of the above
5-	d-non of the above
6-	c-oxaloacetate
7-	c- lactate
8-	e- ATP
9-	b- Lactate