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Metabolism

Metabolism

Definition : It is the sum total of anabolism and catabolism. ***or***
It may be defined as a series of specific biochemical reaction occuring within living organism, catalysed by enzymes, co-enzymes, co-factors and governed by hormones and vitamins.

It consists of anabolism and catabolism.

i. ***Anabolism*** : The synthesis processes or the energy consuming processe those convert small molecules into bigger form for binding up of storage, structural or functional material are collectively known as anabolism.

The chain of reacton of anabolism are :

i. Synthesis of glycogen from glucose- glycogenesis
ii. Lipid synthesis-lipogenesis.
iii. Protein synthesis.
iv. ketone body synthesis.
v. Urea synthesis.
vi. Cholesterol synthesis.

ii. ***Catabolism*** : The breakdown processes or the processes that convert bigger molecules into smaller form for the supply of energy are collectively known as catabolism.

The chain of reaction of catabolisms are :

i. Break down of glycogen into glucose-glycogenolysis.
ii. Break down of glucose- glycolysis.
iii. Break down of lipid-lipolysis.
iv. Protein catabolism.

Steps of catabolism : Catabolism takes place in three broad stages-

First stage : This is the preparatory stage by which the simple fragments derived from complex food stuff, pass into the metabolic platform.

Second stage: It includes the sequence of biochemical reactions, through which the end products of 1st stage are transformed into active acetate or Alpha-ketoglutarate or oxaloacetic acid. About 30% of the total energy is liberated during these reaction.

Third stage : It includes the sequence of biochemical reactions, through which, the end products of second stage are oxidized into CO_2, H_2O & energy. It liberates about 70% of total energgy.

Importance of metabolism : The proximate principles of food are oxidized, producing carbondioxide, water and energy. A part of this energy is stored and rest is utilized for-

i. Protein synthesis and growth of the body.
ii. Muscular activity.
iii. Secretion by the glands.
iv. Maintenance of membrane potential by nerve and muscle fibres.
v. Absorption of food from gastrointestinal tract.

Free energy

The amount of energy liberated by complete oxidation of a food is called free energy of food. Free energy is usually expressed in terms of calories per mole of food substances. For instance, the amount of free energy liberated by oxidation of 1 mole of glucose (180 gms glucose) is 6,86,000 calories.

(Ref. Guyton 11th edition)

Coupled energy

The amount of energy liberated by oxidation of a food with cellular oxygen which energy is not in free but coupled with systems responsible for various physiological function. Energy in the form of ATP, GTP, ADP etc.

Bond energy (energy rich compounds)

Inside the body there are some organic molecules, mostly phosphates or S-H groups which actively participate in metabolic reactions, which has the capacity to take energy during synthesis and liberate energy during their breakdown. The energy which remain in this form is called bond energy.

Though called bond energy, the energy is not located in the bond, but they remains in the structural configuration of the molecules.

Advantage of bond energy : The advantage of bond energy is such that the phosphate group can be directly transferred to another organic moleule along with transfer of energy, which results in the formation of another energy rich phosphorylated molecule.

Exergenic reaction

In the intermediary metabolism of carbohydrate, protein and fat the reaction in which energy is liberated in coupled form or which is useable for the body.

Endergenic reaction

During metabolic change the reaction in which the liberated energy is not useable, for the body is called endergenic reaction.

High energy substances

The substances which liberates large amount of energy (10-12 kcal/mol) when hydrolysed are known as high energy substance e.g ATP, ADP, creatinine phosphate, phosphopyruvic acid, acetyl phosphate, GTP etc.

High energy sbustance or phosphate compounds : The high

energy phosphate bond is designated as pH. The high energy phosphate compounds help in-

i. Transfer of phosphate group easily without loss of much energy.
ii. The energy is stored for utilization in the synthetic process.
iii. Help in oxidative phosphorylation essential for many substances in the body.
iv. When break down, a part of energy is converted into heat & help in thermal balance.

***Adenosine triphospate** (ATP)*

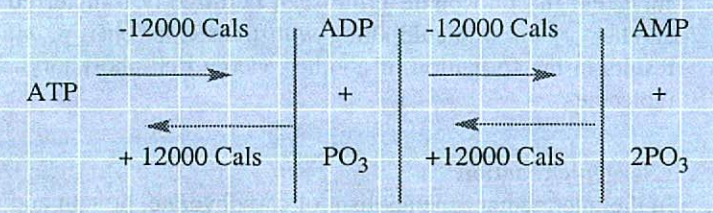
i. *Definition* : ATP (Adenosine triphosphate) is a high energy phosphate compound which is the storage form of energy in the body.

ii *Composition* :
   a. Adenine
   b. Ribose
   c. Phosphates.

iii. *Sources* :
   a. Oxidative phosphorylation
   b. Glycolysis
   c. TCA cycle.

iv. *Functions* : It helps in
   a. Synthetic process
   b. Muscular contraction
   c. Nerve conduction
   d. Active transport
   e. Secretion by the gland.

v. *Role of ATP in metabolism* : Removal of each phosphate radicle liberates 12000 cals of energy. After loss of one phosphate from ATP the compound becomes ADP and after lossing of the second phosphate radicle the compound becomes AMP.



Low energy substances

The substances which liberate relatively small amount of energy (2-3 kcal/mol) on hydrolysis are known as low energy substance e.g Glucose 1-P, Glucose 6-P, Fructose 1-6 diphosphate etc.

Active acetate

The acetyl derivatives of high energy compounds are known as active acetate. When Co-A reacts with acetic acid, acetyl Co-A is formed which is rich in energy.

Q. ***Why Acetyl Co-A is called "active acetate"?***

Ans. Acetyl CoA has a much energy content than acetic acid, So, acetyl Co-A is called active acetate i.e. formation of 1 molecle of acetyl Co-A compound equivalent to the formation of 1 molecule of ATP.

Intermediary metabolism

It is the sum total of those sepecific chemical changes that takes place for a particular type of food from its absorption upto energy yeild.

Example : Glucose → Glycogenesis → Glycogenolysis → Glycolysis → TCA cycle → CO_2, H_2O & energy.

Phosphorylation

The term phosphorylation includes all chemical reactions in the body which require combination of phosphoric acid.

Dephosphorylation : The term includes all chemical reactions in the body where phosphoric acid is dissociated from the compound.

Importance of phosphorylation : Physiological importance of phosphorylation are-

A. *In relation to carbohydrate :*
   i. Absorption of carbohydrate through intestinal mucosa and reabsorption of glucose from renal tubules are helped by phosphorylation.
   ii. Formation of glycogen from glucose & breakdown of glycogen into glucose in the liver & muscle also takes place through phosphorylation.

B. *In relation to fat :*
   i. During absorption of fats; neutral fats & phospholipids are synthesized in the absorbing epithelium by phosphorylation with the help of enzyme phosphorylase.
   ii. Liver synthesized phospholipid specially lecithin.
   iii. Kephalin or cephalin, one of the phospholipids is built up by phosphorylation which initiates blood clotting.
   iv. Each cell can synthesize its own phospholipids locally by phosphorylation.

C. *In relation to protein :*
   i. All the phosphoproteins (Nucleo-protein, caseinogen) are synthesized with the help of phosphorylation.

D. *Miscellaneous :*
   i. Phosphorylation takes an important part in tissue oxidation during which proteins, fats and carbohydrates are finally broken down.
   ii. Some member of Vitamin-B group are phosphorylated compounds, e.g thiamine pyrophosphate.
   iii. Formation of bone i.e deposition of inorganic phosphoric acid compounds occur by phosphorylation.

Control of phosphorylation :

1. Enzymes : The enzymes that take part in this process are phosphorylase, phosphatase etc.
2. Hormones :
   a. Adrenal cortex : Glucocorticoids inhibit phosphorylation.

b. Anterior pituitary : By ACTH it exerts a superior control on phosphorylation, through adrenal cortex.

3. Inorganic Salts : Na^+ may have some effect on phosphorylation.

Q. ***How glucose is phosphorylated?***

Ans. Immediately upon entry into cells, glucose is phosphorylated by ATP. Phosphorylation is performed by the enzyme, glucokinase.

$$\text{Glucose} \xrightarrow[\text{ATP}]{\textit{Glucokinase}} \text{Glucose-6-Phosphate.}$$

Phosphorylation of glucose is almost completely irreversible except in liver cells, renal tubule and intestinal epithelial cells.

Oxidative phosphorylation

Detinition : Oxidative phosphorylation is a coupled reaction where there is oxidation of the substrate as well as phosphorylation i.e. synthesis of ATP from ADP & Pi.

Indication of oxidative phosphorylation : It indicates that during biological oxidation energy is released which is captured in the form of high energy phosphate bond.

Site : Mitochondria

Types :

1. *Substrate level phosphorylation* : Here formation of high energy phosphate takes place on the substrate without undergoing into the respiratory chain.

   Example :

   i. Conversion of phosphoenol pyruvate by *phosphoenol pyruvate kinase* - the release of energy by the hydrolysis of high energy bond of phosphate is utilized for the synthesis of ATP.

   ii. Succinyl CoA → Succinate

   GDP + Pi → GTP ⇢ ATP

2. *Respiratory chain level phosphorylation* : Here formation of high energy phosphate takes place as a result of transfer of H^+ and electrons through the respiratory chain to oxygen.

   Examples :

   i. Isocitrate is converted to oxalosuccinate

   ii. Succinate is converted into fumerate etc.

Mechanism of oxidative phosphorylation :

The exact mechanism of oxidative phosphorylation has not been elucidate. However three proposed mechanism are-

1. *Chemical coupling hypothesis* : Transfer of electrons produces a covalent high energy intermediate compound, which acts as precursor of ATP.

2. *Chemiosmotic coupling hypothesis* : During oxidation H^+ (protons) are produced in the respiratory chain, which are ejected out of the inner mitochondrial membrane. Accumulation of proton on outer face of the inner mitochondrial membrane produces an electro-chemical potential difference, which forces the membrane bound ATP synthetase to generate ATP from ADP + Pi.

3. *Conformational coupling hypothesis* : In this hypothesis it is presumed that energy released during electron transfer is conserved in the conformational changes of the molecules which drive the generation of ATP.

Cyclic AMP

i. *Definition* : It is a cyclic nucleotide. Chemically it is 3', 5'-adenosine monophosphate.

ii. *Synthesis* : It is synthesized in the cell from ATP under the influence of adenyl cyclase in the presence of Mg ions.

iii. *Degradation* : It is degraded in the tissues by its conversion to 5'-AMP in a reaction catalyzed by the phosphodiesterase.

iv. *Biomedical importance* :

   a. It acts as second messenger for many polypeptide hormones.

   b. It activates or inactivates the enzymes that catalyze key reactions of metabolism.

Biological oxidation

Oxidation : Chemically oxidation is defined as the removal of electrons.

$$Fe^{++} \xrightarrow{e^-} Fe^{+++}$$

Oxidation means :

i. Combination with O_2 or

ii. Removal of H^+ or dehydrogenation

Reduction : Loss of O_2 and addition of H^+ or electrons is known as reduction.

Biological oxidation : The oxidation in the living tissue cells is called biological oxidation.

Characteristics of biological oxidation :

i. It is the step wise degradation of metabolites for the generation of energy.

2. Final phase of biological oxidation is the utilization of O_2 and production of CO_2 by the tissues in the process of cellular respiration.

3. Enzymes, co-cnzymes and co-factors are needed for biological oxidation.

4. Enzymes of biological oxidation are highly specific, but co-enzymes & co-factors are not specific for a reaction.

The enzymic actions are activated by the presence of some accessory substances. When these accessory substances are single ions (Mg^+, PO_4^-), they are called *co-factors*, but when they are complex non-protein organic molecules, they are known as *co-enzymes*.

Difference between oxidation and biological oxidation :

Oxidation occurs only one step of reaction but biological oxidation occurs through several steps of reaction.

Oxido-reductases

Enzymes of biological oxidation :

Enzymes that carry out biological oxidation are known as oxido-reductases. They are classified into five groups.

1. *Oxidase :* They catalyses the removal of H_2 but uses only O_2 as hydrogen acceptor.
   Example : Cytochrome oxidase. phenolase, lactase etc.
2. *Aerobic dehydrogenase :* They catalyses the removal of H_2 but uses either O_2 or methyle blue, (artificial substances) as hydrogen aceptor.
   Example : D-amino-acid oxidase, L-amino-acid oxidase
3. *Anaerobic dehydrogenase :* They catalyses the removal of hydrogen but can not use O_2 as hydrogen acceptor.
4. *Hydroperoxidase :* These enzymes use H_2O_2 as their substrate.
5. *Oxygenase :* They catalyzes the direct transfer and incorporation of oxygen into substrate molecule.

Respiratory chain (electron transport chain) :

Respiratory chain is a series of catalyst in the inner mitochondrial membrane that are involved in the transport of reducing equivalents- H^+ or electron from the substrate to molecular oxygen to form water with the generation of ATP.

Organization (components) : There are four classes of components :

1. Flavoproteins (containing FMN & FAD)
2. Ubiquinone (coenzyme Q)
3. Iron-sulfur protein (FeS)
4. Cytochromes (cyt a, b, c).

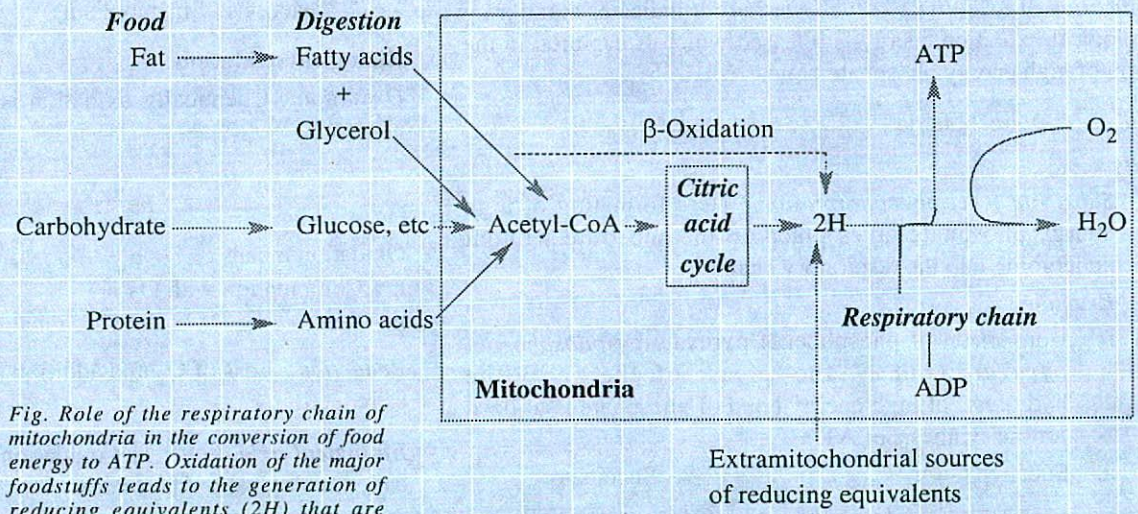


Fig. Role of the respiratory chain of mitochondria in the conversion of food energy to ATP. Oxidation of the major foodstuffs leads to the generation of reducing equivalents (2H) that are collected by the respiratory chain for oxidation and coupled generation of ATP.

(Ref: Harper's Illustrated Biochemistry 26th Edition, Page-93)

The components of the chain constitute five seperate protein-lipid respiratory chain complexes (complex I, 11, III, IV & V).

Cytochrome c is the only soluble cytochrome and together with *co-enzyme Q*, seems to be a more mobile component of the respiratory chain connecting the fixed complexes. Co-enzyme Q transports electron from the complexes I & II to complex III; cytochrome c transports electron from complex III to complex IV.

(Ref: Harper's Illustrated Biochemistry 26th Edition; Page-92)

Sites of ATP production :

i.	NAD	FMN
ii.	Cyt b	Cyt c_1
iii.	Cyt a	Cyt a_3

Importance :

The carriers in the respiratory chain are arranged so that spontaneous flow of electrons to oxygen is ensured. This is important to release energy by electron flow through respiratory chain.

Electron transport from NADH to oxygen produce 3 moles of ATP.

Respiratory chain co-enzymes : *Respiratory chain co- enzymes are* :

1. *FMN (flabin mononucleotide) & FAD (flabin adenine dinucleotide)* : These are the biologically active form of riboflavin (vitamin B_2).

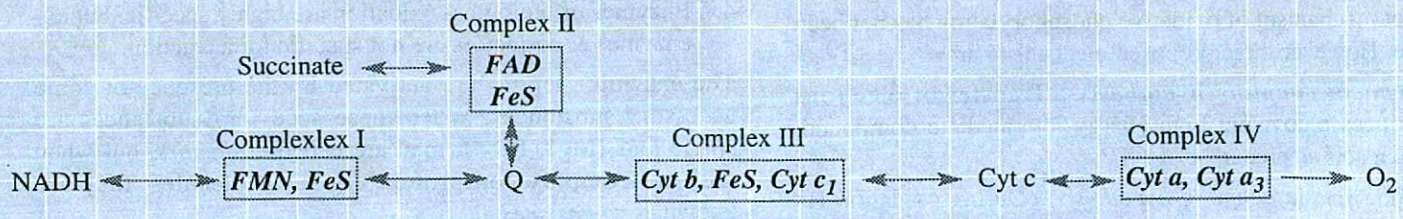


Fig: The respiratory chain complexes.

(Ref: Harper's Illustrated Biochemistry 26th Edition, Page-96)

Function of FMN :

a. FMN is bound to complex I and accepts 2 hydrogen atoms becoming $FMNH_2$.

b. FAD is bound to complex II and accepts 2 hydrogen atoms becoming $FADH_2$.

2. *Co-enzyme Q* : It is a quinone derivative with a long isoprenoid tail. It is also called ubiquinone because it is ubiquitous in biological systems.

   Function : It collects the reducing equivalents from the more fixed Fp complexes to the cytochromes.

3. *Iron-sulfur protein (FeS)* : It is an additional component in respiratory chain. It is nonheme iron.

   Function : It is associated with Fp (metallo-Fp) & with cytochrome b. The sulfur & iron take part in oxidoreduction between flavin &co-enzyme Q which involves a single e^- change, the Fe atom undergoing oxidoreduction between Fe^{++} & Fe^{+++}.

4. *Cytochromes* : They are the heam containing proteins. They act as e^- carriers in the respiratory chain. They are of 5 types :
   i. Cyt b
   ii. Cyt c_1
   iii. Cyt c
   iv. Cyt a
   v. Cyt a_3.

   Function : These cytochromes take part in electron transport between Fe^{++} & Fe^{+++}.

(Ref: Harper's Illustrated Biochemistry 26th Edition; Lippincott's Illuetrated Reviews of Biochemistry 23rd Edition)

Arrangement of the components of respiratory chain : The components of the respiratory chain are arranged in order to increasing redox potential.

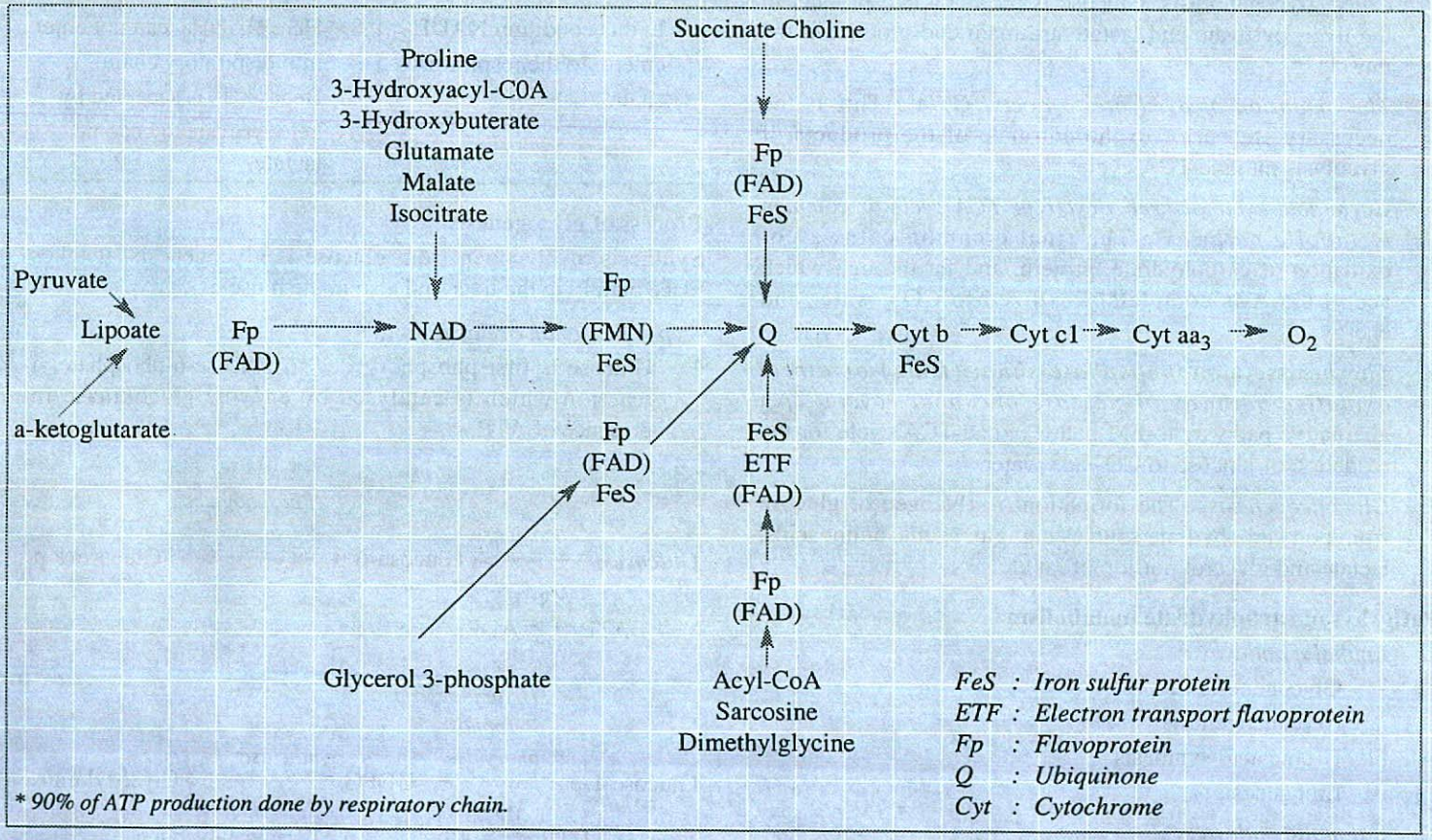


Fig. Components of the respiratory chain in mitochondria, showing the collecting points for reducing equivalents from important substrates. FeS occurs in the sequences on the O_2 side of Fp or Cyt b. *(Ref: Harper's Illustrated Biochemistry 26th Edition, Page-94)*

Carbohydrate Metabolism

The end products of carbohydrate digestion are mainly glucose (80%) and small amount of fructose and galactose. Galactose is converted exclusively in liver into glucose; fructose is converted both in the liver and muscles into glucose. So, glucose is the main form in which carbohydrate is metabolized in the body.

(Ref. Guyton & Hall 11th edition & Wright's)

Intermediary metabolism of carbohydrate : Intermediary metabolism of carbohydrate may be subdivided as follows :

1. *Glycolysis :* The oxidation of glucose to pyruvate or lactate by Embden-Meherhop pathway (EM).
2. *Glycogenesis :* Synthesis of glycogen from glucose is called glycogenesis.
3. *Glycogenolysis :* Breakdown of glycogen into glucose is called glycogenolysis. Glucose is the main end product in the liver, pyruvate and lactate are main end product in the muscle.
4. *The oxidation of pyruvate to acetyl Co-A :* This is the necessary step prior to the entrance of the products of glycolysis into the TCA cycle.
5. *Citric acid cycle or kreb's cycle or TCA cycle or common metabolic pathway :* The final common pathway of oxidation of carbohydrate, protein, and fat through which acetyl Co-A is completely oxdized to CO_2, water and energy.
6. *The hexose monophosphate shunt (HMS) or direct oxidative pathway or pentose phosphate cycle :* An alternative pathway to EM pathway and TCA cycle for the oxidation of glucose to CO_2 and water.
7. *Gluconeogenesis :* The formation of glycogen or glucose from non carbohydrate sources e.g. glucogenic aminoacids, lactate and glycerol portion of fat etc.

Pathways of carbohydrate metabolism

a. *Anabolic pathway :*
   i. Glycogenesis
   ii. Neoglucogenesis
   iii. Uronic acid synthesis
   iv. Lipogenesis.

b. *Catabolic pathway :*
   i. Glycolysis
   ii. Oxidation of pyruvate to acetyl CoA
   iii. TCA cycle
   iv. Glycogenolysis
   v. HMP shunt.

Q. ***Which metabolism gives more ATP and why?***

Ans. Aerobic pathway of carbohydrate metabolism give more ATP than anaerobic pathway. Because during aerobic pathway the $NADH_2$ enter into respiratory chain and oxidized. So, aerobic pathway give more ATP than anaerobic pathway.

Aerobic glycolysis

Breakdown of glucose in presence of oxygen is known as aerobic glycolysis.

Anaerobic glycolysis

Breakdown of glucose in absence of oxygen is known as anaerobic glycolysis.

Difference between aerobic and anaerobic glycolysis

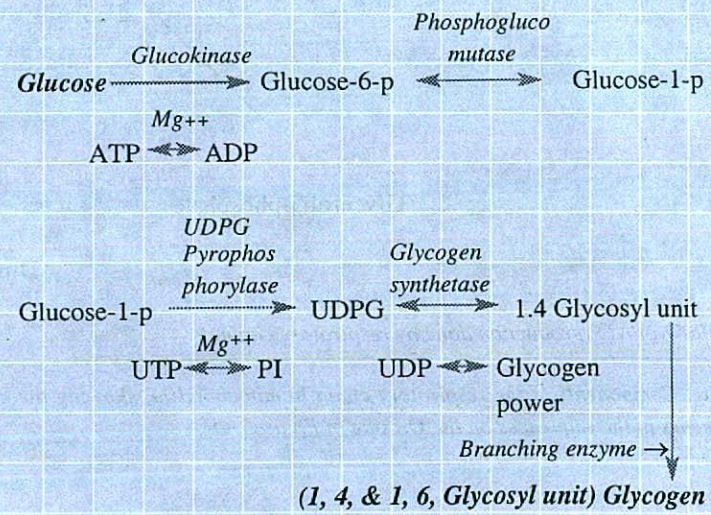
Aerobic	*Anaerobic*
1. In this condition pyru vate enters into the TCA cycle for oxidation.	1. In this condition pyruvate is converted into lactate in presence of lactatedehydro genase.
2. Here 8 molecules of ATP is formed.	2. Here 2 molecules of ATP is formed.
3. In this condition $NADH_2$ enter into the respiratory chain.	3. Here $NADH_2$ can not enter into respiratory chain. Becuse H^+ is accepted by ketopyruvate to become lactate.

Process of glycogenesis

Synthesis of glycogen from glucose is glycogenesis. It takes place mainly in the liver and also in the muscle.

Steps of reaction are as follows :

1. Glucose is first phosphorylated to glucose-6-phosphate, a reaction which is catalysed by enzyme glucokinase in presence of ATP.



2. Glucose-6-phosphate is then converted into glucose-1-phosphate, catalysed by the enzyme phosphoglucomutase.
3. Next glucose-1 phosphate reacts with urindine triphosphate (UTP) to form the active nucleotide uridine diphosphate

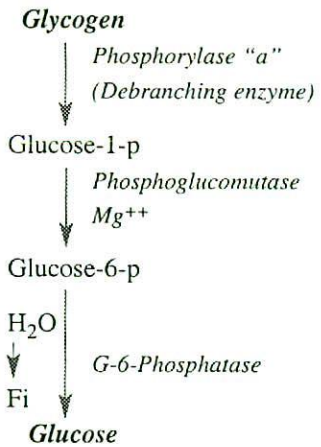
glucose (UDPG). This reaction is catalysed by enzyme UDPG pyrophosphorylase.

4. By the action of glycogen synthetase, the C_1 of the activated glucose of UDPG forms a glycosidic bond with C_4 of a non-reducing terminal glucose residue of pre-existing glycogen. Thus uridine diphosphate (UDP) is liberated.
5. A second enzyme called branching enzyme (amylo 1-4 & 1-6 transglucosidase) acts on glycogen and transfer a part of 1-4 chain of a neighbouring chain to form a 1-6 linkage and helps in glycogen synthesis by establishing a branch point in the molecule.

(Ref: Harper's Illustrated Biochemistry 26th Edition, Page-145)

Glycogenolysis

The process of breakdown of glycogen to reform glucose is known as glycogenolysis.



Steps of glycogenolysis : This takes place under the influence of an enzyme called phosphorylase. Phosphorylase exists in an inactive form phosphorylase "b" *(dephosphophosphorylase)*. It is activated to phosphorylase "a" (activated form) by the action of some hormones in the following way :

Epinephrine activates adenyl cyclase → Which causes the formation of cylic AMP → Which activates protein kinase regulator protein → Which activates protein kinase →Which activates phosphorylase b kinase → Which converts phosphorylase "b" into phosphorylase "a". This phosphorylase "a" promotes degradation of glycogen and the process of glycogenolysis proceeds in the following way :

a. The activatd phosphorylase (Phosphorylase "a") acts on glycogen and converts into glucose-1-phosphate.
b. The glucose-1-phosphate is converted into glucose-6-phosphate catalysed by the enzyme phosphoglucomutase.
c. In liver and kidney (but not in muscle) glucose-6-phosphate is converted into glucose under the enzymatic action of glucose-6-phosphatase. This glucose then passes into the blood.

(Ref: Harper's Illustrated Biochemistry 26th Edition, Page-145)

Glycolysis *(under aerobic condition)*

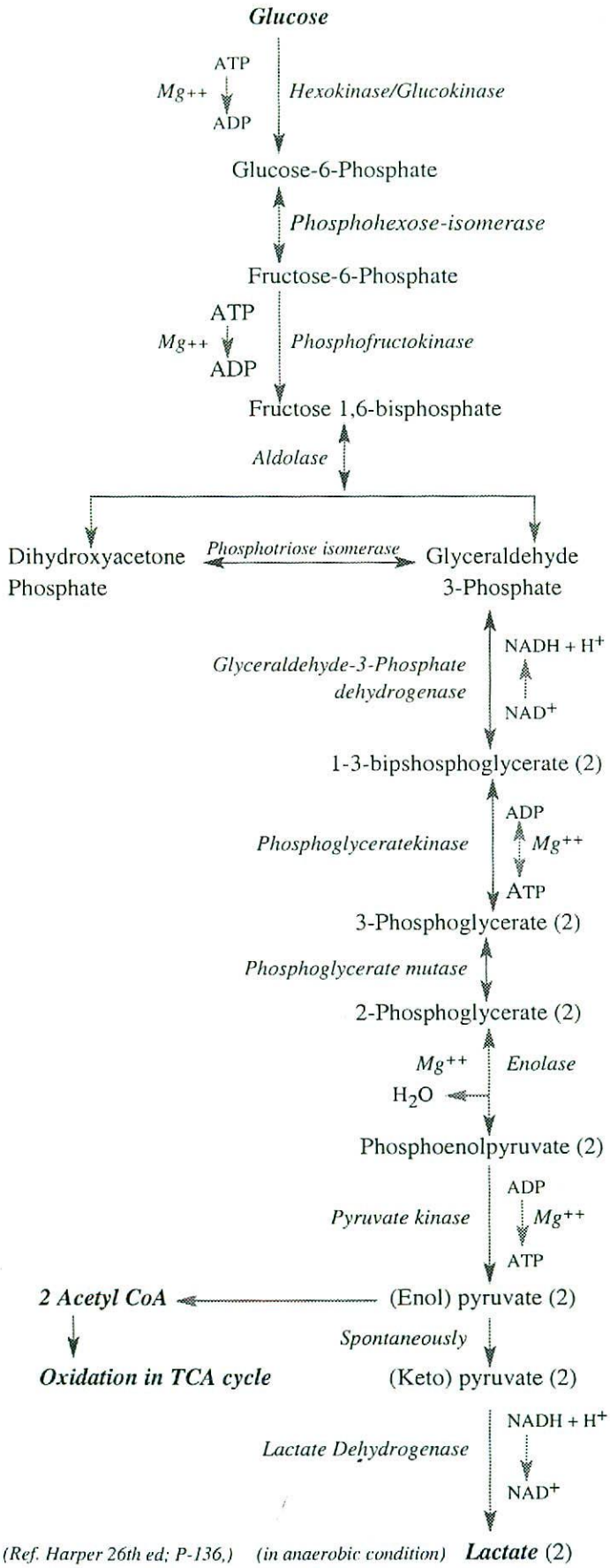
Gycolysis means oxidation of glucose or glycogen to pyruvate or lactate by Embden-meherhop pathway. It occurs in cytoplasm not in the nucleus or mitochondria.

Factors in glycolysis :

1. Substrate : Glucose
   i. Aerobic glycolysis : Pyruvate (2)
   ii. Anaerobic glycolysis : Lactate
2. Site : Allmost all cell
3. Compartment : Cytoplasm
4. Nature of pathway : Catabolic.

Steps of reaction :

1. Glucose is phosphorylated to glucose-6-phosphate. This is accomplished by the enzyme hexokinase and by an additional enzyme in the liver called glucokinase. ATP is required as phosphate donar and Mg^{++} acts as a co-factor and reacts as Mg-ATP complex. One high energy phosphate bond of ATP is utilized and ADP is produced.
2. Glucose-6-phosphate is converted to fructose-6-phosphate by phospho-hexose isomerase.
3. Fructose-6-phosphate is phosphorylated to fructose-1-6 bisphosphate, catalysed by the enzyme phosphofructo-kinase. ATP and Mg^{++} is required for phosphorylation.
4. Fructose 1-6-bisphosphate is split by aldolase into two triose phosphate- glyceraldehyd-3-phosphate and dihydroxy-acetone phosphate. These two triose phosphate are interconverted by the enzyme phosphotriose isomerase.
5. Glyceraldehyde-3 phosphate is then oxidized by glyceraldehyde-3 phosphate dehydrogenase into 1-3 diphosphoglycerate and because of the activity of the 6-phosphotriose isomerase the dihydroxyaectone phosphate is also oxidized to 1-3 diphosphoglycerate and glyceraldehyde 3 phosphate. Here removal of H^+ occurs which is accepted by NAD.
6. 1-3 diphosphoglycerate then denotes one high energy phosphate to ADP to convert it into ATP and it itself is transformed into 3 phosphoglycerate. This reaction is catalysed by phosphoglycerate kinase. Here Mg^{++} acts as a co-factor.
7. 3-phosphoglycerate is converted to 2-phosphoglycerate by the enzyme phosphoglycerate mutase.
8. 2-phosphoglycerate is convert to phosphoenol pyruvate by lossing 1-molecule of water (Dehydration). This reaction is catalysed by the enzyme enolase.
9. Phosphoenol pyruvate is converted into enolpyruvate by *pyruvatekinase*. In this reaction phosphoenolpyruvate losses PO_4 group which is accepted by ADP.
10. Then enolpyruvate spontaneously converted into ketopyruvate. This is another nonequilibrium reaction that



is accompanied by considerable loss of free energy as heat and must be regarded as physiologically irreversible.

Under aerobic conditions, pyruvate is taken up into mitochondria, and after conversion to acetyl-CoA is oxidized to CO_2 by the citric acid cycle.

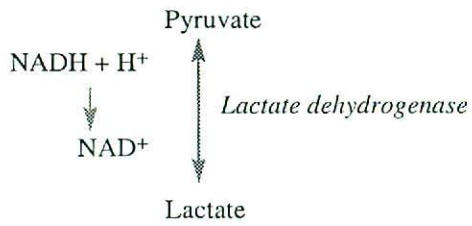
(Ref. Harper 26th ed; page-136, 137)

Glycolysis under anerobic condition

(Anaerobic pathway for carbohydrate metabolism) :

See steps of aerobic glycolysis and then add the following paragraph : In anaerobic conditions, the reoxidation of NADH by transfer of reducing equivalents through the respiratory chain to oxygen is prevented. Pyruvate, which is the normal end product of glycolysis under aerobic condition is reduced under anaerobic condition to lactate by NADH, the reaction being catalysed by "*lactate dehydrogenase*".

The reoxidation of NADH via lactate fromation allows glycolysis to proceed in the absence of oxygen by regenerating sufficient NAD^+ for the reaction catalysed by *glyceraldehyde-3-phosphate dehydrogenase.*



2 molecules of ATP is formed by glycolytic pathway under anaerobic condition.

N.B. In RBC : Always anaerobic glycolysis.

(Ref. Harper's 26th ed; page-139)

Q. ***How many ATP is formed during glycolysis under aerobic condition..***

Ans. 8 ATP is formed in glycolytic pathway under areobic condition. The following reactions are involved in the formation of ATP.

ATP used in glycolytic pathway :

Reaction catalyzed by	*Mechanism*	*Molecules of ATP*
1. Hexokinase/ Glucokinase.	At substrate level	1 ATP
2. Phosphofructo-kinase	At substrate level	1 ATP
		Total = 2 ATP

ATP gained in glycolytic pathway :

Enzymatic level	*Mechanism*	*Molecules of ATP*
1. Glyceraldehyde -3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	6 ATP

2. Phosphoglycerate kinase	Phosphorilation at substrate level	2 ATP
3. Pyruvate kinase	Phosphorilation at substrate level	2 ATP
	Total	= 10 ATP

So, ATP formed in aerobic glycolysis (10 - 2) = 8 molecules.

Q. ***How many ATP will formed from one mole of glucose in glycogen by glycolytic pathway?***

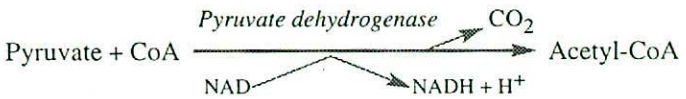
Ans.

3 moles of ATP will produced anaerobically from 1 mole of glucose in glycogen by glycolytic pathway. Because free glucose entering into the cell must be phosphorylated by 1 mole of ATP before it can begin to be split. Where as this is not true to glucose derieved from glycogen because it comes from glycogen in phosphorylated state without the expenditure of ATP.

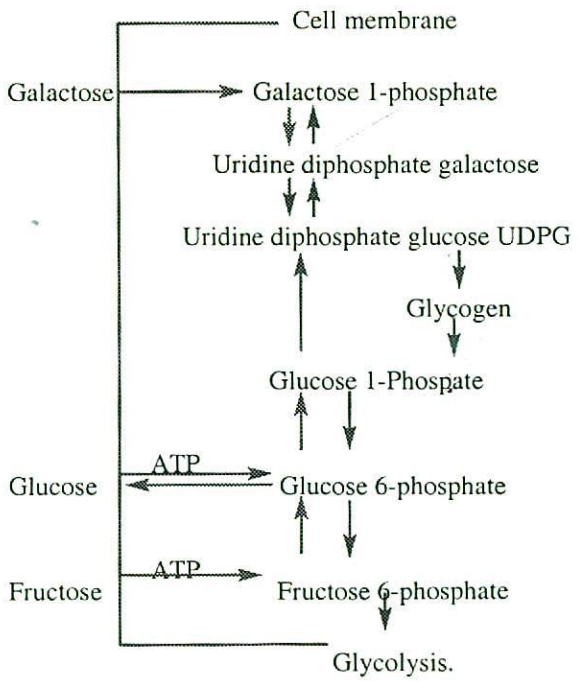
(Ref. Guyton & Hall 11th edition)

Q. ***How pyruvate is converted into Acetyl-CoA?***

Ans. Before pyruvate can enter the cytric acid cycle it must under goes oxidative decarboxylation to form acetyl-CoA. This reaction is catalysed by several enzymes collectively designated as *"pyruvate dehydrogenase complex"*.



Interconversion of glucose, fructose and galactose



(Ref. Guyton & Hall 11th edition)

Pyruvate

Source of pyruvate :

i. From glucose by glycolytic pathway
ii. From amino acid by transamination.
iii. From fatty acid by beta-oxidation.

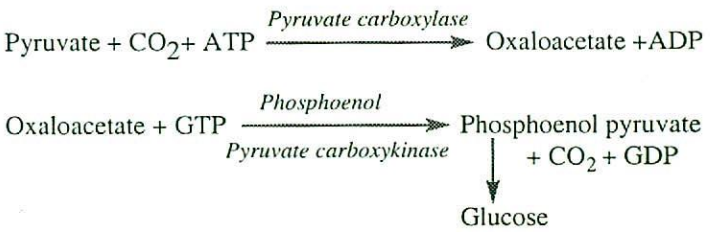
Fates of pyruvate : Pyruvate is converted into-

i. Lactic acid under anaerobic condition.
ii. Acetyl CoA by oxidative decarboxylation.
iii. Oxaloacetic acid by carboxylation.
iv. Glucose in TCA cycle by gluconeogenesis
v. Alanine by transamination.

Q. ***How pyruvate is converted into glucose?***

Ans. Pyruvate is converted into glucose by gluconeogenesis reaction.

i. An enzyme pyruvate carboxylase in presence of ATP, biotin and CO_2 converts pyruvate to oxaloacetate, it occurs in mitochondria.
ii. Second enzyme "phosphoenol pyruvate carboxykinase" catalyses the convertion of oxaloacetate to phosphoenol pyruvate. High energy phosphate in the form of GTP is required and CO_2 is liberated.
iii. Phosphoenol pyruvate then converted into glucose by series of reactions.

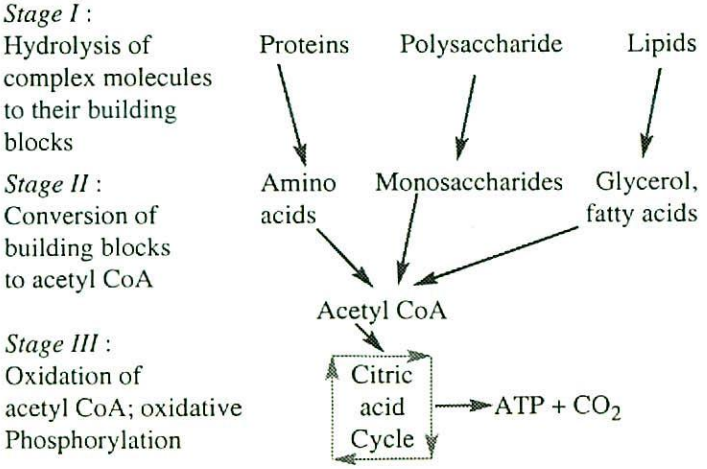


(Ref. Harper's 26th edition)

Source and fate of acetyl CoA

Source :

i. Carbohydrate metabolism (Glycolysis).
ii. Fat metabolism (Beta-oxidation).
iii. Protein metabolism (Transamination).



(Ref. Lippincott's Illustrated Reviews 3rd Edition)

Fate of acetyl-CoA :

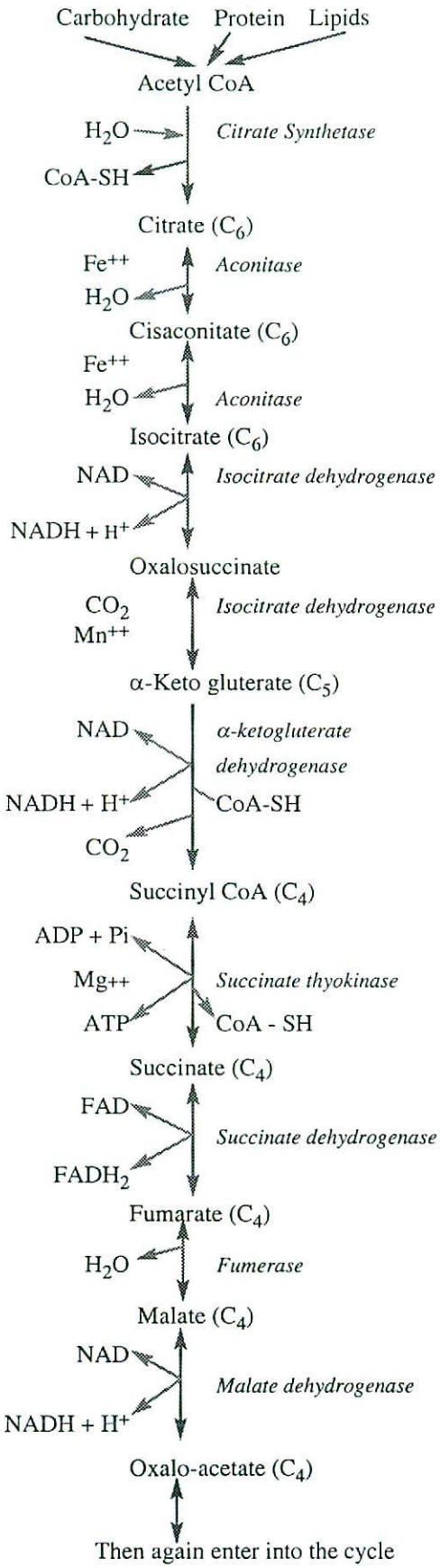
a. Utilization in TCA cycle to produce CO_2, water and energy.
b. Acetyl CoA gives rise to cholesterol synthesis.
c. Acetyl CoA gives rise to fatty acid synthesis.
d. Formation of ketone bodies.
e. Synthesis of steroid substances.
f. Acetyl CoA undergoes acetylation reactions.

TCA cycle

1. Substrate : CO_2, H_2O, energy
2. Site : Mitochondrial
3. Compartment : Mitochondria
4. Nature of pathway : Amphibolic.
5. *ATP production from 1 mole of glucose* :
   - i. Glycolysis : 8 ATP (10-2)
   - ii. 2 Pyruvate to 2 acetyl CoA : 6 ATP
   - iii. TCA cycle (2 acetyl CoA) : 24 ATP (12x2)
   - *Total* = *38 ATP*

Reactions of the citric acid cycle : Before pyruvate can enter the TCA cycle it must undergo oxidative decarboxylation to form acetyl-CoA with the help of pyruate dehydrogenase complex.

1. *Condensation* : The acetyl-CoA condenses with oxaloacetate to form citrate, a reaction catalyzed by a condensing enzyme citrate synthetase. This is the first reaction of citric acid cycle.
2. *Dehydration and rehydration* : Citrate first by a process of dehydration is converted into cisaconitate. Which again by a process of rehydrations transformed into isocitrate. The enzyme aconitase catalyzes both the steps.
3. *Dehydrogenation* : Isocitrate undergoes dehydrogenation in presence of isocitrate dehydrogenase to form oxalosuccinate. NAD acts as hydrogen acceptor and converted into $NADH_2$.
4. *Decarboxylation* : An enzyme isocitrate dehydrogenase in presence of Mn^{++} removes CO_2 from oxalosuccinate which is thus converted into α-ketogluterate.
5. *Oxidative decarboxylation* : Next α-ketogluterate under goes oxidative decarboxylation and converted into succinyl CoA. This reaction is catalyzed by α- ketogluterate dehydrogenase complex.
6. *Succinyl CoA* is converted into succinate by the enzyme succinate thiokinase (Succinyl CoA synthetase). This reaction require GDP or IDP which is converted in presence of inorganic phosphate (IP) to GTP or ITP.
7. *Dehydrogenation* : Succinate is converted into fumerate by succinate dehydrogenase. The hydrogen is transfered directly to FAD-converting it into $FADH_2$.



(Ref. Harper 26th edition; page-130, 131)

8. *Hydration* : Under the influence of fumarase water is aded to fumerate and converted it into malate.
9. *Dehydrogenase* : By this process malate is converted into oxaloacetate by malate dehydrogenase, a reaction requiring NAD^+. Oxaloacetate then again enter into the cycle.

(Ref. Harper's 26th ed; page 130)

Q. ***How many ATP is formed in citric acid cycle?***

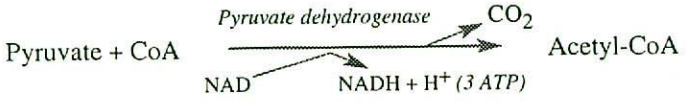
Ans.

TCA Cycle starts from Acetyl-CoA and one molecule of Acetyl CoA forms 12 molecule of ATP. They are formed in the following way :

Reaction catalyzed by	*Method of high-energy p production*	*Number of high-energy p formed per molecule of glucoes*
1. Isocitrate dehydrogenase	Respiratory chain oxidation of NADH	3
2. α ketogluterate dehydrogenase	Respiratory chain oxidation of NADH	3
3. Succinate thiokinase	Phosphorylation at substrate level	1
4. Succinate dehydrogenase	Respiratory chain oxidation of $FADH_2$	2
5. Malate dehydrogenase	Respiratory chain oxidation of NADH	3
		Net = 12 ATP

ATP formed in TCA cycle :

1. From Acetyl CoA (1) : 12 ATP.
2. From pyruvate (1) : 15 ATP (3 ATP + 12 ATP)



(Ref. Harper's 26th edition, page-133)

Generation of high-energy phosphate in the catabolism of glucose :

A. ***Glycolysis*** :

ATP used in glycolytic pathway :

Reaction catalyzed by	*Method of high-energy p production*	*Number of high-energy p used per molecule of glucoes*
1. Hexokinase/ Glucokinase.	At substrate level	1 ATP
2. Phosphofructo-kinase	At substrate level	1 ATP
		Total = 2 ATP

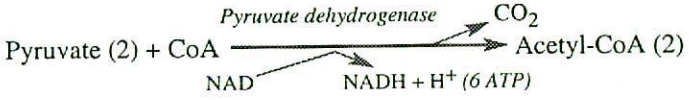
ATP gained in glycolytic pathway :

Reaction catalyzed by	*Method of high-energy p production*	*Number of high-energy p formed per molecule of glucoes*
1. (2) Glyceraldehyde -3-phosphate dehydrogenase	Respiratory chain oxidation of 2NADH	6 ATP
2. Phosphoglycerate kinase	Phosphorilation at substrate level	2 ATP
3. Pyruvate kinase	Phosphorilation at substrate level	2 ATP
Total		= 10 ATP

So, In aerobic glycolysis : (10 - 2) 8 molecules of ATP + 2 molecules of pyruvate is formed.

B. ***Citric acid cycle*** :

(1 molecule of glucose = 2 pyruvate = 2 Acetyl CoA)



Reaction catalyzed by	*Method of high-energy p production*	*Number of high-energy p formed per molecule of glucoes*
1. Pyruvate dehydro genase	Respiratory chain oxidation of *2 NADH*	6
2. Isocitrate dehydro genase	Respiratory chain oxidation of *2 NADH*	6
3. α-ketogluterate dehydrogenase	Respiratory chain oxidation of *2 NADH*	6
4. Succinate thiokinase	Phosphorylation at substrate level	2
5. Succinate dehydrogenase	Respiratory chain oxidation of *2* $FADH_2$	4
6. Malate dehydrogenase	Respiratory chain oxidation of *2 NADH*	6
		Net = 30

Total per mole of glucose under aerobic conditions : 38 (8+30)
Total per mole of glucose under anaerobic conditions : 2

(Ref. Harper's 26th edition; Page 143)

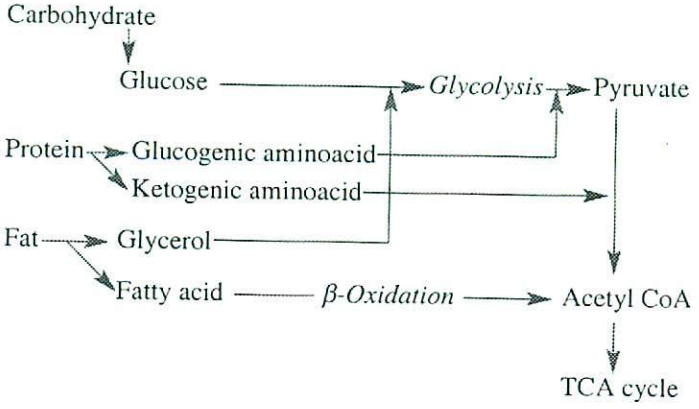
Q. ***Show diagramatically and explain that TCA cycle is the common metabolic pathway.***

Ans. *Explanation* :

1. Glucose formed by carbohydrate digestion is undrgone through EM pathway and form pyruvic acid (Ketoacid) which then form acetyl CoA by oxidative decarboxilation reaction.

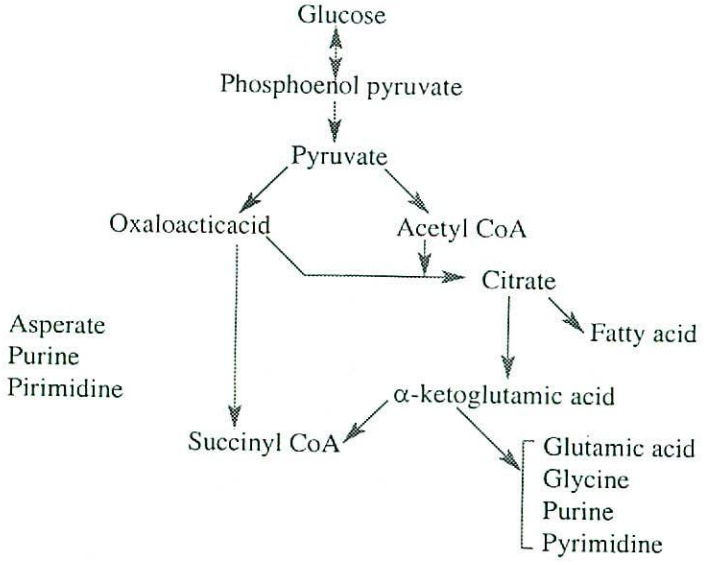
2. Amino acid, comes from hydrolysis of protein, passes through deamination and transamination and form keto acid. This keto acid then form acetyl Co-A.

Table : TCA cycle as common metabolic pathway :



3. Fatty acid, formed by the digestion of fat, enters into beta-oxidation and form acetyl Co-A.
4. Acetyl CoA comes from the metabolism of carbohydrate, protein and fat; then couples with oxaloacetic acid and initiate TCA cycle.

TCA cycle as amphibolic (catabolic + anabolic) :



HMP-shunt

Hexose monophosphate shunt pathway is an alternative pathway to Embden-Meherhop pathway and citric acid cycle for the oxidation of glucose to CO_2 and water. It is called hexose monophosphate shunt because it strarts from glucose-6-phosphate and all the reaction is phosphate related.

Importance of HMP-shunt :

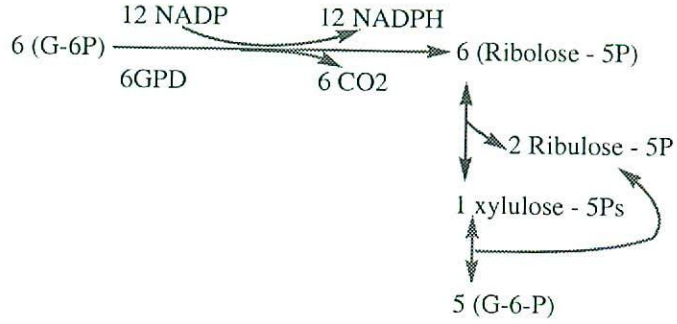
1. To produce ribose for nucleotide synthesis
2. To produce NADPH for-
   i. Reductive synthesis of fatty acid, steroid, cholesterol
   ii. Detoxifing function of liver
   iii. Anti-oxidant activity
   iv. Bacterial killing in phagocytosis
3. Alternate pathway for glucose oxidation.

Factors of HMP-shunt :

i. Site : Cytosol

ii. 10% of body glucose is oxidised by HMP-shunt.

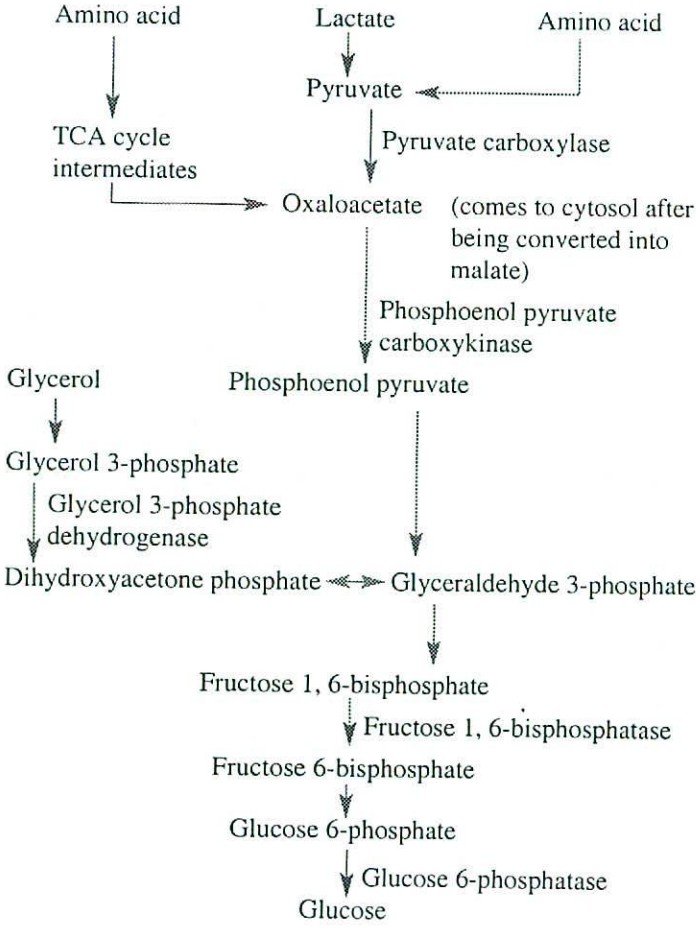
iii. Steps :



Neoglucogenesis / gluconeogenesis

Definition : The formation of glycogen or glucose from non carbohydrate sources e.g. glucogenic aminoacids, lactate and glycerol portion of fat etc.

Steps of gluconeogenesis :



Factors of gluconeogenesis :

1. Substrate : Glucogenic aminoacid, glycerol, lactate, pyruvate, intermediates of TCA cycle.
2. Product : Glucose
2. Site : Liver (90% of total), kidney (10% of total)
3. Compartment : Primarily cytoplasm
4. Nature of pathway : Anabolic.

Most important substrate of neoglucogenesis is glucogenic aminoacids.

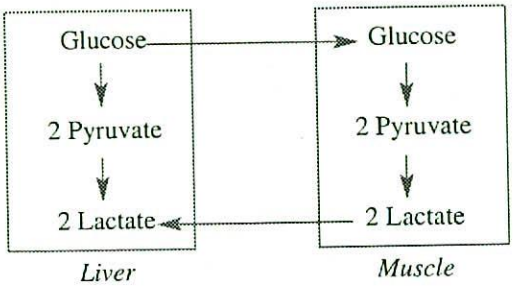
Biomedical importance :

1. To fulfill the need of glucose of the body because every cell needs a particular amount of basal glucose to maintain TCA cycle.
2. Some tissues & cells are solely dependant on glucose. Gluconeogenesis supports the supply of glucose to these obligate glucose consuming cells - neurons, RBC, renal medulla & gonads.
3. Maintenance of blood glucose concentration during fasting and starvation & in between meals.

Oxaloacetatc is the key intermediate of gluconeogenesis because it is the final intermediate of TCA cycle that can be converted to glucose by gluconeogenesis.

Cori cycle or *lactic acid cycle*

Lactic acid produced in the muscle reaches the liver through blood, where it is converted into glucose by gluconeogenesis, which again becomes the source of energy for utilization. Thus this continues and is called " Coti cycle or lactic acid cycle."



Lactate disposal :

i. Lactate production : 750-1500 meq/day
ii. Lactate disposal : Mostly by cori-cycle.

Sources of blood glucose

There are two important sources, which give rise to glucose in the body :

1. *Dietary source :* Carbohydrates of food after digestion and absorption are the chief source.
2. *Sources within the body :*
   a. Glycogenic compound : Glycogenic amino acid, glycerol of fat & intermediate products of glycolytic pathway; by process of gluconeogenesis.
   b. Liver glycogen : By process of glycogenolysis.

End product of glucose :

1. In aerobic glycolysis : Pyruvate.
2. In anaerobic glycolysis : Lactate.

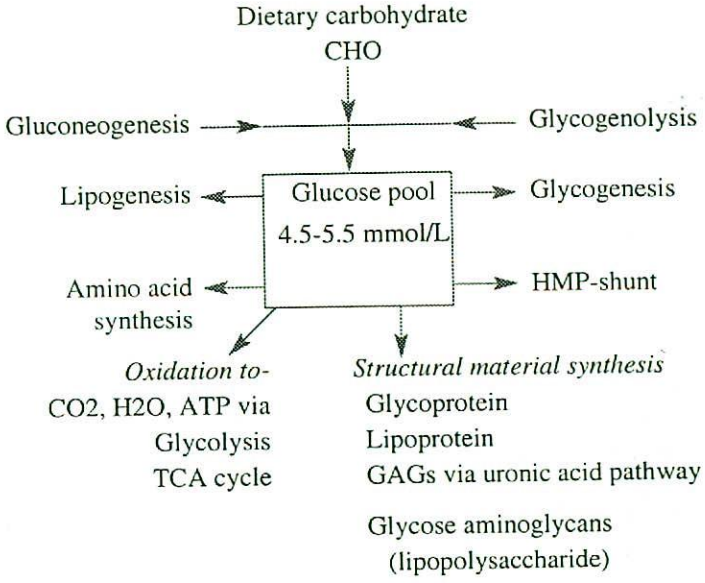
Fate of glucose :

Possible fates of glucose are as follows-

1. Provision of energy to body; About 4.1 kcal/gm.
2. Storage as glycogen in the liver and muscle.
3. Convertion into fat and stores in the fat depots.
4. Transamination of some intermediate products of glucose break down to form amino acid.

(Ref. Wright's)

Carbohydrate pool



Importance of glucose :

1. It is the major source of energy in the body.
2. Certain tissues like nervous tissues can utilize only carbohydrates (glucose).
3. It supplies energy to muscles even without the presence of O_2, when no other source of energy can be utilized.
4. It supplies raw materials for formation of milk suger in mammary gland.
5. It is lipogenic.
6. It is the chief source of high energy phosphate.

Sources of liver glycogen

The possible sources of liver glycogen are-

1. *From carbohydrate & related substances* :
   a. End products of carbohydrate digestion e.g. Glucose, fructose & galactose
   b. The intermediate products of carbohydrate metabolism e.g-Pyruvic acid, lactic acid etc.
2. *From Proteins* : The anti-ketogenic amino acid can readily form glucose through TCA cycle or reversible glycolytic pathway or both. These are-Glycine, alanine, aspartic acid, glutamic acid etc.

3. *From fats* : The glycerol part of fat is converted into glucose from which glycogen may be derived.

Fate of liver Glycogen :

1. It converts into glucose when blood glucose level tends to fall.
2. It break downs & liberates energy in starvation, exercise etc.

Source of muscle glycogen

1. Blood glucose
2. From lactic acid : Which is produced in the muscle during its contraction. The major part (4/5th) of lactic acid produced during exercise is reconverted into glycogen..

Fate of muscle glycogen :

It can only breaks down into lactic acid.

Q. ***Why muscle glycogen cannot be the source of blood glucose?***

Ans.

Muscle glycogen cannot be the source of blood glucose, because muscle lacks an enzyme glucose-6-phosphatase; necessary for conversion of glucose.

Q. ***How muscle glycogen is utilized for energy?***

Ans.

In glycogenolysis, the muscle glycogen is firstly converted into glucose-1-phosphate then glucose-6-phosphate. Since the muscle tissue contains no *glucose-6-phosphatase*, the glucose-6-phoshate converted into fructose-6-Phosphate which enters either the EM pathway or hexose monophosphate shunt pathway and utilized for energy, thus lactic acid is formd.

Difference between liver and muscle glycogen

Liver glycogen	*Muscle glycogen*
1. Amou nt : 100 mg	1. Amount : 400 mg
2. Liver glycogen is utilized for maintenance of blood sugar.	2. Muscle glycogen is not available for maintaining of blood sugar.
3. Turn over rate- rapid	3. Turn over rate slow.
4. As fuel not used by the liver.	4. Used by the muscle.
5. Source : From carbohydrate, lactic acid as well as muscle, from protein and fats.	5. Source : From blood glucose, muscle, lactic acid, not from proteins.
6. Liver glycogen can converted into glucose.	6. Muscle glycogen can not converted into glucose.
7. Glycogen breakdown is mostly upto glucose.	7. Glycogen breakdown is upto pyruvate and lactate.
8. Almost mobile.	8. Less mobile.

Q. ***Why CHO are stored in the form of glycogen?***

Ans. Glycogen is a suitable form to store carbohydrate because-

1. It is insoluble & so exerts no osmotic pressure.
2. It can not diffuse from its storage sites.
3. It is readily broken down to glucose in the liver to enter the blood stream.
4. It has a higher energy level than a corresponding weight of glucose.

(Ref. Wright's 13th page-451)

Glucostatic function of liver

Definition : Maintenance of normal blood glucose level by liver during the tendancy of hypoglycemia or hyperglycemia is called glucostatic function of liver.

I. *In hyperglycemia* :
   a. Increase glycogenesis
   b. Increase lipogenesis.

II. *In hypoglycaemia* :
   c. Increase glycogenolysis
   d. Increase gluconeogenesis.

Importance of glucostatic function :

1. Fuel supply to neuron and RBC
2. Minimum basal glucose supply to every cell
3. Prevent cerebral impairment
4 Prevent ketosis, (-) N_2 balance.

Mechanism :

a. *Glycogenolysis* : During fasting when both the blood concentration and insulin level fall, the liver releases glucose from its stored glycogen by the process of glycogenolysis. By this process, the liver can maintain normal blood glucose level upto 12-18 hours.
b. *Gluconeogenesis* : Liver can store 100 gm glycogen. In starvation, at least 12-18 hours after meal, all stored glycogen is broken down into glucose. Then gluconeogenesis starts ie. glucose is formed from non-carbohydrate substances. Thus normal blood glucose level is maintained.
c. *Glycogenesis* : After the ingestion of carbohydrate, when blood glucose level rises to a high level and insulin secretion increases, as much as is $^2/_3$ of glucose absorbed from the gut is stored immediately as glycogen in liver and muscle. Thus blood glucose concentration remains normal.
d. *Lipogenesis* : Extra glucose i.e if glucose in blood after 100 gm glycogen in liver and 400 gm glycogen in muscle which is their highest capacity of storage, is converted into fat by the process of lipogenesis.

Hypoglycaemia :

Blood glucose less than 55 mg%.

1. Fasting hypoglycaemia e.g in cirrhotic patient
2. Reactive or poestprandial- common.

Hypoglycaemia protection by :

i. Glucagon
ii. Cortisol
iii. Catecholamine
iv. Growth hormone.

Factors maintaining blood glucose level

1. Humoral
2. Hormonal
3. Nervous.

Humoral factors :

It includes :

i. Role of liver : The liver acts as an important *blood glucose buffer system.*
   a. When blood glucose level rises to very high, after a meal, 2/3 of glucose absorbed from gut is almost immediately stored in liver as glycogen.
   b. When blood glucose level falls, liver releases glucose back into blood due to presence of glucose-6-phosphatase.
   c. When glycogen reservoir diminishes, liver enhances gluconeogenesis.

ii. Role of muscle :
   a. During increase blood glucose level : Promotes glycogenesis and oxidation of glucose in muscle.
   b. During decrease blood glucose level : Muscle glycogen supplies glucose to blood by cori cycle. It can not serve as a direct source of blood glucose during hypoglycaemia due to absence of glucose-6-phosphatase.
   c. During exercise : It promotes entry of glucose into muscle cell thus decreasing blood glucose level.

iii. Role of kidney :
   a. During increase blood glucose level : When blood glucose level exceeds the renal threshold level, excess glucose is excreted in urine, thus decreases blood glucose level.
   b. During decrease blood glucose level : Kidney possesses gluconeogenesis, thus increases blood glucose level.

Hormonal factors :

i. Insulin : Decrease blood glucose level
   a. Increase glucose uptake from blood to all cells of the body
   b. Increase glycolysis
   c. Increase glycogenesis
   d. Decrease glycogenolysis
   e. Decrease gluconeogenesis

ii. Glucagon : *Increase blood glucose level*
   a. Increase glycogenolysis
   b. Increase gluconeogenesis

iii. Glucocorticoids : *Increase blood glucose level*
   a. It stimulates the gluconeogenesis in liver,
   b. It decreases the peripheral utilization of glucose.
   c. It decreases the transport of glucose to the periphery.

iv. Growth hormone : *Increase blood glucose level*
   a. Decrase uptake and utilization of glucose

v. Epinephrine : *Increase blood glucose level*
   a. Increase glycogenolysis

vi. Thyroid hormones : *Increase blood glucose level*
   a. Increase glycogenolysis
   b. Increase gluconeogenesis

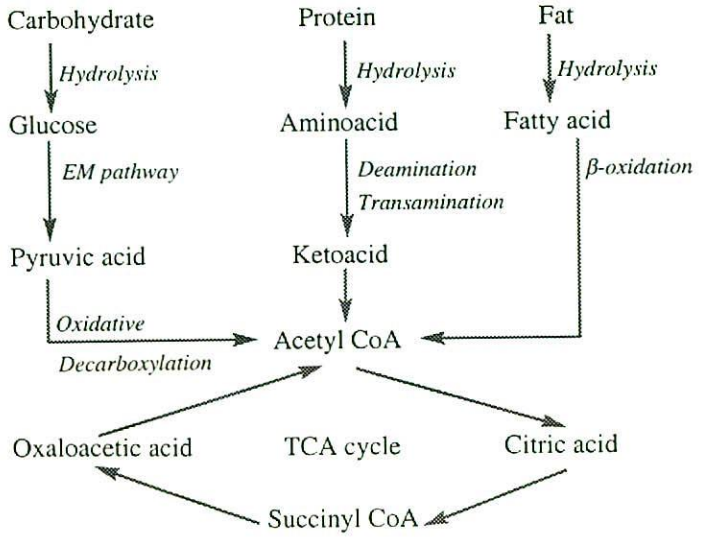
vii. ACTH : *Increase blood glucose level*
   a. Increase gluconeogenesis.

Nervous factor :

1. Hunger sensation : Raises blood glucose level by taking food during hypoglycemia.
2. Satiety : Inhibits hunger centre, there by decreases blood glucose.

N.B. *Neuron, RBC : 100% dependent for nutrition on glucose.*

TCA cycle as common metabolic pathway :



Protein metabolism

Definition : The metabolism of proteins means metabolism of amino acids.

Body can not produce essential amino acid from glucose but can do non-essential amino acid.

Factors f protein metabolism :

i. Protein turn over : 1-2% (300-400 gm/day) of the total body protein (12-14 kg).

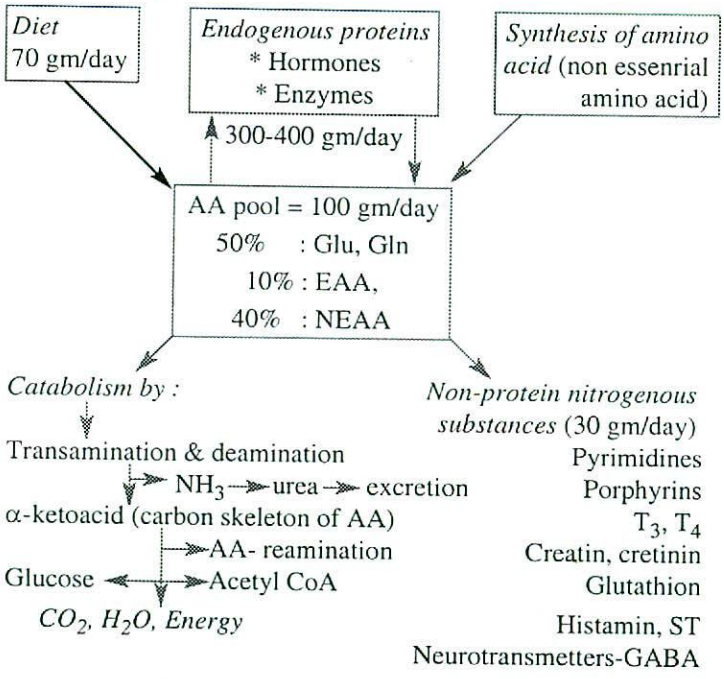
ii. Site : All cells of the body

iii. Compartment : Lysosomes

iv. Every amino acid converts to corresponding ketoacid by deamination.

v. Every ketoacid converts to corresponding amino acid by amination.

Amino acid pool :



Amino acid pool : There are constant exchange of amino acid from tissue to blood and other body fluids and reversely from body fluid to the tissue, which is known as amino acid pool. The inflow and out flow are so balanced that the dynamic equilibrium of amino acid is maintained nicely.

Inflow of amino acids into pool :

1. Amino acid from dietary proteins.
2. Break down of tissue protein.
3. Synthesis of amino acids by transamination of keto acids in the liver.
4. Reabsorption of amino acids from renal tubules.

Out flow of amino acids from the pool :

1. Synthesis of protein in the body.
2. Synthesis of essential non-protein nitrogen compound.
3. Synthesis of non-protein substances.
4. Oxidation to yeild energy.

Fate of amino acids :

By the process of deamination, amino acid is broken into two parts-

i. Nitrogenous parts

ii. Non-nitorgenous part.

i. ***Fate of nitrogenous part (ammonia)*** : Ammonia undergoes the follwing fates :
   1. Formation of urea.
   2. Formation of ammonium salts.
   3. Formation of new protein.
   4. It may be used for synthesis of various nitrogenous substances such as creatinine, purine, uric acid, lacithine etc.
   5. It may be excreted through urine.

ii. ***Fate of non-nitrogenous part*** : This part of amino-acid has the following fate-
   1. Helps in the formation of glucose by neoglucogenesis.
   2. Give rise to ketone bodies.
   3. Sulphur and phosphorus compounds are formed and are excreted.

Amino acids

1. ***Glucogenic amino acid*** :
   i. *Definition* : Amino acids whose catabolism yields pyruvate or one of the intermediates of the citric acid cycle are called glucogenic amino acids.
   ii. *Example* :
      a. *Essential glucogenic amino acids* : Arginine, histidine, methionine, threonine, valine.
      b. *Non-essential glucogenic amino acids* : Alanine, asparagine, asparate, cysteine, glutamate, glutamine, glycine, proline, serine.
2. ***Ketogenic amino acid*** :
   i. *Definition* : Amino acids whose catabolism yields either acetoacetate or one of its precursors- acetyl CoA or acetoacetyl CoA are called ketogenic amino acids.
   ii. *Example* :
      a. Essential ketogenic amino acids : Leucine, lysine.
3. ***Both glucogenic and ketogenic amino acid*** :
   i. *Definition* : Amino acids whose catabolism yields either pyruvate/one of the intermediates of the citric acid cycle or acetoacetate/one of its precursors- acetyl CoA/ acetoacetyl CoA are called both glucogenic and ketogenic amino acid.
   ii. *Example* :
      a. Essential both glucogenic and ketogenic amino acids : Isoleucin, phenylalanine, tryptophan

b. Non-ssential both glucogenic and ketogenic amino acids : Tyrosine.

(Lippincott's Illustrated Reviews of Biochemistry 2nd Edition; P 246)

Process of protein metabolism

Proteins are catabolized in the following ways :

i. Transamination.
ii. Oxidative deamination.
iii. Ammonia transport.
iv. Reactions of the urea cycle.

(Ref. Harper's 26th edition)

Transamination

Interconversion of a pair of amino acid and a pair of ketoacid. That is conversion of one amino acid to the corresponding ketoacid with simultaneous conversion of another keto acid to an amino acid is called transamination. In this reaction amino group of an amino acid is transfered to the keto group of ketoacid resulting in the formation of corresponding ketoacid and amino acid. This reaction is catalized by transaminase or aminotansferase enzyme. It is reversible.

Alanine + alpha ketogluteric acid $\xrightarrow{\text{Transaminase}}$ Pyruvic acid + Glutamic acid

(Ref. Harper's 26th edition)

Factors of transamination :

i. Compartment : Cytoplasm
ii. Sites : Liver, skeletam muscle, cardiac muscle
iii. Direction : Bi-direction
iv. Enzyme : Transaminase.
v. Amino acid : Any ketoacid can participate in transamination i.e-
   a. α-keto gluterate ⇢ Glutamate
   b. Pyruvate ⇢ Alanine
   c. Oxaloacetate ⇢ Asperate.

Importance of transamination :

1. This is an easy way of forming new amino acids (Non essential) from α-keto acids.
2. It provides a link between fat, carbohydrate and protein metabolism, as the keto acids come from the metabolic cycle.
3. It serves a missing link in the pathway of ammonia formation.
4. By this process-amino acids synthesized in the liver pass to the amino acid pool.

Oxidative deamination

Deamination means removal of amino group from an amino acid thus transforming it to the corresponding ketoacid. As oxidation occurs simultaneously by dehydrogenation, it is also called oxidative deamination. It occurs in the liver. Here amino acid oxidase is involved.

1. *Step.1 :* Amino acid is first dehydrogenated by amino acid oxidase to form imino acid. Hydrogen ions are carried by FAD and converted into $FADH_2$.
2. *Step.2 :* The iminoacid then spontaneously hydrolyzed to the corresponding ketoacid with liberation of ammonia.

Aminoacid (alanine)
FAD ↓ — *Aminoacid oxidase*
$FADH_2$ ↓
Iminoacid + H_2O
↓
Keto acid (pyruvic acid) + NH_3

Factors of oxidative deamination :

i. Compartment : Cytoplasm
ii. Sites : Liver and kidney
iii. Substrate : Amino acid
iv. Products : NH_3 and keto acid
iv. Enzyme : Amino acids oxidase, glutamate dehydrogenase

Importance :

i. Formation of NH_3 which serves as a source of nitrogen in urea synthesis.
ii. Formation of a-keto acid which can enters into the central pathway of urea synthesis.

(Ref. Harper 26th edition)

Fates of keto acid

The ketoacids resulting from deamination of amino acids are mostly oxidized with energy release. This oxidation involves conversion of keto acid into an intermediate product that enters into the TCA cycle and is degraded e.g ketoacid obtaining from alanine is pyruvic acid, obviously it can be cornverted into acetyl CoA and then into fatty acids. Also 2 molecules of aetyl CoA condense to form acetoacetic acid.

Fate of ammonia in the body

I. *Ammonia undergoes the follwing fates* :
   1. Formation of urea.
   2. Formation of ammonium salts.
   3. Formation of new protein.
   4. It may be used for synthesis of various nitrogenous substances such as creatinine, purine, uric acid, lacithine etc.
   5. It may be excreted through urine.

II. *Metabolic disposal of ammonia : Ammonia is disposed from the body mainly by 2 processes* :
   i. *By excreting NH_3* : Ammonia is secreted into the renal tubules derived from glutamine in the presence of glutaminase.

ii. *By forming urea* : The free ammonia which is produced in the body, circulated in the blood is taken up by the liver and converted into urea by urea cycle. Then it is excreted through the kidney into urine. Brain tissue can form urea but this does not play a significant role in ammonia removal.

Mechanism of synthesis of urea *or* urea cycle

Liver is the only site of the urea formation. The process of urea synthesis divided into five different phases.

1. *Synthesis of carbamyl phosphate* : Condensation of 1 molecule each of ammonia, CO_2 and phosphate (derived from ATP) forms carbamyl phosphate. This reaction is catalyzed by carbamyl phosphate synthetase. 2 molecules of ATP hydrolyzed during this reaction provide the driving force for synthesis of two covalent bonds-
   a. The amide bond.
   b. Mixed carboxylic acid-phosphoric acid anhydride bond.

   In addition Mg^{++} and a carboxylic acid, N-acetylglutamate is required.
2. *Synthesis of citruline* : Transfer of a carbamyl moiety from carbamyl phosphate to ornithine, forming citruline and Pi. This reaction is catalyzed by L-ornithine transcarbamylase of liver mitochondria.
3. *Synthesis of arginine succinate* : In this reaction aspartate and citruline are linked together via amino group of asparate. This reaction requires ATP and arginino-succinate synthetase.
4. *Cleavage of arginino succinate to arginine and fumerate* : The arginino-succinate then splits into arginine and fumarate catayzed by the enzyme arginino succinase.
5. *Cleavage of arginine to ornithine and urea* : This reaction completes the urea cycle and regenerates ornithine. Here hydrolytic cleavage of the guanidine group of arginine is catalyzed by arginase. Ornithine is produced and at the same time urea is formed. This ornithine further couples with carbamyl phosphate.

(Ref. Harper 26th edition)

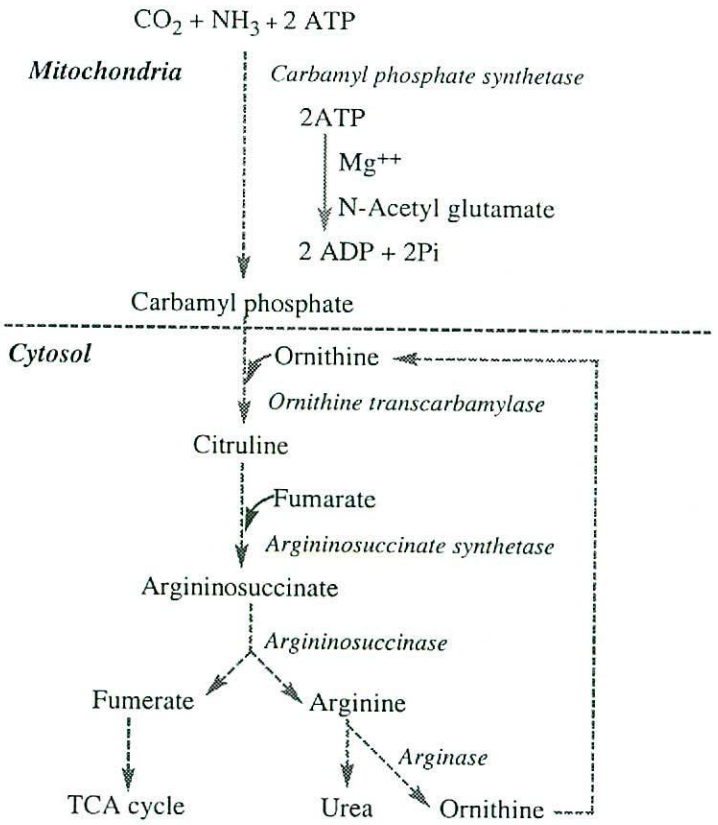


Fig. Diagram of urea synthesis (urea cycle).

Nitrogen balance

Nitrogen balance is the state of the body in which the intake and output of nitrogen are same i.e intake = excretion.

i. *Daily intake of nitrogen* : 16 gm/day
   N_2 content of protein is 16%.
   Normal protein intake/day : 75-100 gm/day
   Intake of nitrogen : about 16 gm/day as protein (16% of protein).

ii. *Daily excretion of nitrogen* : 16 gm/day
   a. Stool and sweat : 2 gm/day
   b. Urine : 14 gm/day
      1. Urea : 85 %
      2. NH_4 : 3 %
      3. Creatinin : 5 % (in fasting increase 15% of total excretion)
      4. Uric acid : 2 %
      5. Undetermined : 5 %

Types of nitrogen balance : It is of two types-

a. *Positive nitrogen balance* : When the intake of protein is more in relation to the nitrogen excretion; then it is called "positive nitrogen balance". i.e growing infant, pregnant mother.
b. *Negative nitrogen balance* : When the excretion of nitrogen is increased than the intake of protein; then it is called negative nitrogen balance i.e kawshiorker, post surgical patient. (-Ve) N_2 balance can be tolerable upto 30%.
c. *Nitrogen equilibrium* : i.e in normal adult.

Importance of nitrogen balance :

1. It reflects the nature of protein utilization in the body and to some extents, an index of physical health.
2. It reflects an index of protein requirement in the body.
3. It measures to some extent of endogenous protein metabolism in the subject.
4. It helps in planning of diet.

Fat metabolism

Blood lipids

i.	Triglycerides	: 35-160	mg%
ii.	Phospholipid	: 145-200	mg%
iii.	Cholesterol	: 150-220	mg%
	a. Cholesterol ester	: 155	mg%
	b. Cholesterol	: 45	mg%
iv.	Free fatty acid (non essential fatty acid)	: 6-16	mg%

Common fatty acids :

1. Saturated fatty acids (50%) : Palmatic acid (16 C), stearic acid (18 C)
2. Unsaturated fatty acids (50%) : Mainly polyunsaturated fatty acid.

Sources of polyunsaturated fatty acids :
i. Fish oil
ii. Vegetable oil except coconut oil, palm oil.

Function : Prevents hypercholesterolaemia.

Plasma level of free fatty acid : 0.1 - 2.0 μeq/ml

Source :

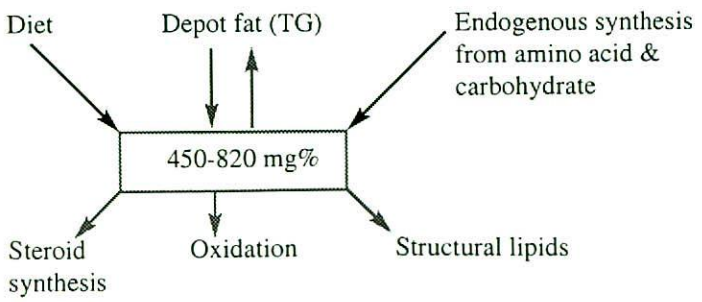
1. Triglycerides of adipose tissue by HSL -ve → In fasting. Well fed → increase insulin → decrease HSL
2. After meal
   i. Chylomicron : By LPL (capillary endothelium of adipose tissue)
   ii. VLDL : By LPL (capillary endothelium of adipose tissue).

   Increase well fed → Increase insulin → Decrease HSL → increase LPL.

Lipid profile

i.	Triglycerides	: < 150	mg%
ii.	Cholesterol	: 150-220	mg%
iii.	HDL	: > 40	mg%
iv.	LDL	: < 130	mg%
v.	LDL : HDL	: 2.5 : 1	
vi.	Cholesterol : HDL	: 3.5 : 1	

Lipid pool : The continuous exchange of blood lipid to the tissue and from tissue to the blood is called lipid pool.



Inflow to blood :

a. Absorped fatty acid & glycerides of the food.
b. By mobilization from depot fat.
c. Synthesis from CHO & proteins.

Out flow from blood :

a. Oxidation in liver
b. Storage in depot fats
c. Formation of structural lipid
d. Formation of milk fat in mammary gland
e. Formation of steroid hormones
f. Formation of lipoprotein complex
g. Formation of ketone bodies.

Factors maintain lipid pool :

1. *Liver* : Liver is predominantly the main organ where synthesis as well as oxidation of lipid goes on side by side.
2. *Endocrine glands* : The anterior pituitary growth hormone and insulin from pancreas greatly affect the lipid pool by affecting metabolism.

Alimentary lipaemia

After a fatty meal, the concentration of plasma lipids begin to rise within 1-2 hours and reaches maximum within 6 hours. This rise of plasma lipids after a fatty meal is termed as alimentary lipaemia.

The chief increase takes place in Neutral fat; phospholipids & cholesterol may also slightly increase.

Sites of fat storage

Large quantities of fat are stored in two major tissues of the body.

i. The adipose tissue
ii. The liver.

Fat depots

Adipose tissue of the body are called fat depots, as they store large quantities of neutral fat.

Depot fat

Definition : The amount of fat that remains stored in the body & is used up during the body need is called depot fat. It constitute about 12% of the total body weight.

Distribution :

i.	Subcutaneous tissue	: 50%
ii.	Perirenal tissue	: 15%
iii.	Mesentry	: 20%
iv.	Omentum	: 10%
v.	Intramuscular connective tissue	: 5%

Composition : Depot fat chiefly composed of the glycerides of various fatty acids and usually Contains 75% of oleic acid, 20% palmitic acid & 5% stearic acid.

Sources :

The depot fat may derived from-

1. Food fat-Chief source
2. Carbohydrate.
3. Proteins.

Functions :

1. Mechanical-
   a. It gives shape to the limbs & body.
   b. It keeps some viscera at position.
   c. Acts as a mechanical buffer aganist injury.
2. Physical : The subcutaneous fat helps in heat regulation.
3. Chemical : It represents stored energy.

Element variable

It represents the fat which disappears during starvation. It is mainly neutral fat.

Element constant

It represents the fat content in the tissue which remains constant even during starvation and can not be reduced without death occuring. It consists mainly of cholesterol and phospholipid.

Antilipotropic factor

Substances which increases the deposition of fat in the liver is called antilipotropic factor.

Example: Cholesterole.

Fate of fat after absorption

After absorption, fat is treated in various ways-

1. It undergoes complete oxidation in the tissues to yeild energy, CO_2 & H_2O. 1 gm of fat oxidized to give 9.3 kcal of heat.
2. Actyl coA an intermediate product, produces aceto acetic acid & contain body component.
3. It is stored in the fat depots (as neutral fat).
4. Fat builds up the structure of many tissues.

(Ref. Wright's)

Metabolic fate of fat

Fats whether derived from food or mobilized from depots, come into the liver. In the liver, with the help of enzyme lipase the fat molecule at first breaks up into fatty acids and glycerol.

Fat $\xrightarrow{Lipase}$ Fatty acid + glycerol.

Fatty acid undrgoes β-oxidation and liberates energy.

Glycerol on oxidation enters into glycolytic pathway as glyceraldehyde.

Difference between element constant & element variable

Element constant (Structural fat)	*Element variable (Depot fat)*
1. It fails to be mobilized during starvation	1. It is mobilized during starvation to meet energy need.
2. It is bound with protoplasm of the cell. So, cannot be extracted with fat solvent.	2. It is present in adipose tissue cells as solvent & can be extracted with fat solvent.
3. It consists mostly of phospholipid & cholesterol.	3. It consists mostly of glycerides, i.e neutral fat.
4. These contain mostly unsaturated fatty acids.	4. These contain mostly saturated fatty acids.
5. It reflects functional activity	5. It is concerned with intermediary metabolism of fat.
6. It can not be shown by histological staining technique.	6. It is possible to show them by staining technique.

Lipotropic factor *(Lipotropins)*

Substances which prevent the deposition of fats and acelerates the rate of removal of fat from the liver, are known as lipotropic factors.

The exact mechanism of action of lipotropic factor is unknown. It is believed that they all help in the synthesis of phospholipid in which form liver fat may be easily mobilized out.

Lipotropic factors are - choline, methionine, betanine, lecithine, inositol, casein & certain other proteins etc.

Beta Oxidation

The process of oxidation of fatty acids is called beta oxidation as the fatty acids are split of successively at betacarbon position i.e. third carbon atom from COOH group. Thus two carbons at a time are removed in the form of acetyl CoA.

Factors of β oxidation :

i. Substrate : Fatty acid
ii. Product : Acetyl CoA
iii. Site : Liver, skeletal muscle, cardiac muscle, adipose tissue
iv. Compartment : Mitochondria
v. No. of acetyl CoA formation from fatty acid :
   Formula : $(^{n}/_{2} - 1)$ number of turns e.g from 16 C palmitic acid → 8 acetyl CoA, by 7 turns.

Stages of beta-Oxidation :

1. *Activation of fatty acid radicle :* In presence of ATP and co-enzyme A, the enzyme thiokinase (Acyl CoA

synthetase) catalyzes the conversion of fatty acid to an active fatty acid or acyl CoA. This is the only step in the complete degradation of fatty acid that requires energy from ATP.

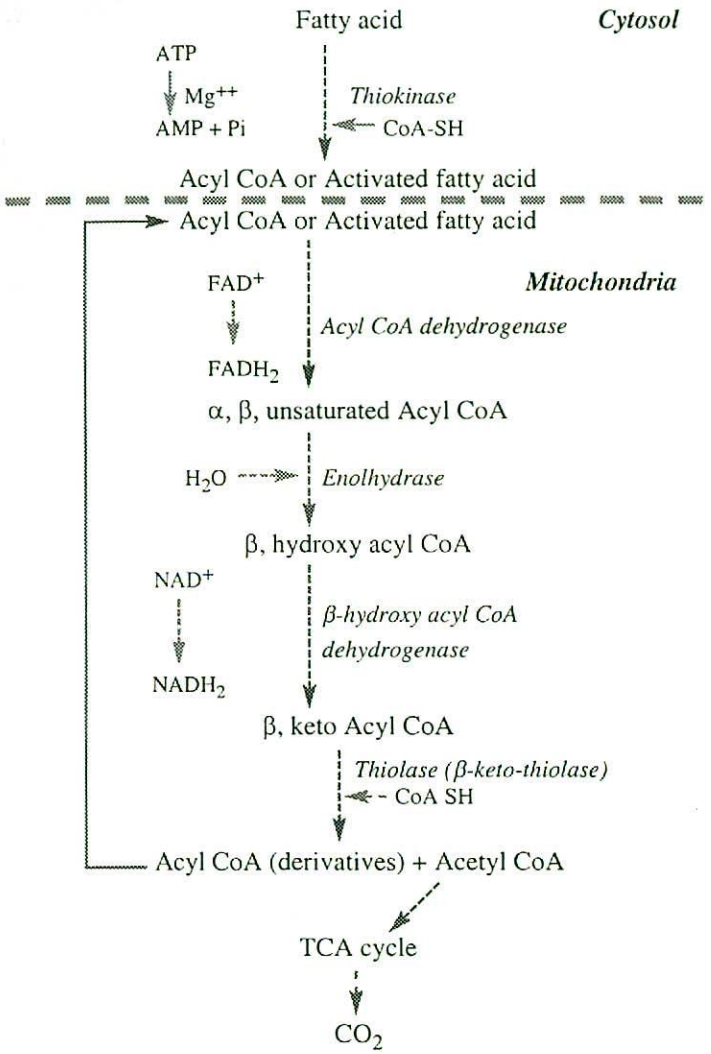


Fig. *Beta-Oxidation of fatty acid.*

2. *Dehydrogenation :* After the formation of acyl CoA, there follows the removal of 2 hydrogen atom from the alpha and beta-carbon (leaving a double bond at this point), catalyzed by acyl CoA dehydrogenase. Thus alpha, beta unsaturated acyl CoA is formed. The co-enzyme for dehydrogenation is flavoprotein.
3. *Hydration :* Then alpha, beta unsaturated fatty acid passes through a process of hydration with the addition of water under the influence of enolhydrase and beta hydroxy acyl CoA is formed.
4. *Dehydrogenation :* There is removal of two hydrogen atoms from beta carbon atoms& beta-keto acyl CoA is produce. NAD acts as a hydrogen acceptor being converted into $NADH_2$. This reaction is catalyzed by beta hydroxy acyl CoA dehydrogenase.
5. *Thiolytic clevage :* Finally beta-keto acyl CoA is split at beta position by thiolase, which catalyzes a thiolytic cleavage involving another molecule of CoA. The product of this reaction are acetyl CoA and an acyl CoA derivative; containing 2 carbons less than the original acyl CoA molecule which underwent oxidation.

   The acyl-CoA formed in the cleavage reaction re-enters the oxidative pathway at reaction number (2) and proceed untill another acetyl-CoA molecule is released. This process is repeated again and again until the entire fatty acid molecule is split into acetyl-CoA. For instance, stearic acid with 18 carbon atoms, nine molecules of acetyl-CoA are formed.

(Ref. Harper 26th edition)

N.B. ***In new born baby*** : Lacking of enzymes of β-oxidation → Gluconeogenesis is imperfect, as gluconeogenesis consumes energy produced in β-oxidation. These babies after long term fasting develop hypoglycaemia → Death (SIDS- sudden infant death syndrome).

Fate of Glycerol

In presence of ATP and glycerol kinase, glycerol is phosphorylated to give glyceraldehyde 3-phosphate (Phospho-glyceraldehyde). This is oxidized to 1-3 diphosphoglycerate which is metabolized by the glycolytic pathway.

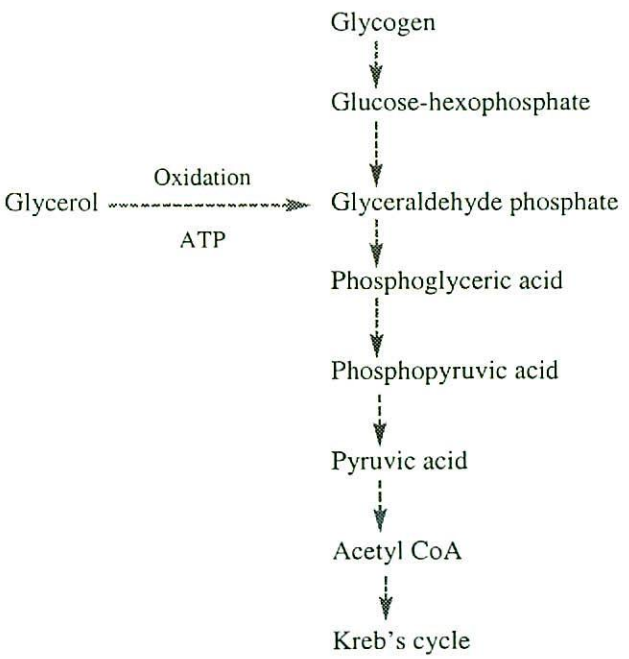


Fig. Metabolic transformation of glycerol.

End product of even number carbon fatty acid

Acetyl CoA :

End products of odd number carbon fatty acid-acetyl CoA & propionyl CoA. Propionyl CoA is formed from three terminal carbon atoms from the methyl end of fatty acids.

Q. ***How many ATP is formed from 1 molecule of stearic acid?***

Ans.

Number of acetyl CoA formation from fatty acid :

Formula : $(^{n}/_{2} - 1)$ number of turns

e.g from 18 C stearic acid 9 acetyl CoA is formed by 8 turns.

One molecule of stearic acid contains 18 carbon atoms. In Beta-Oxidation of stearic acid produces :

i. 9 molecules of acetyl-CoA
ii. 8 molecules of $FADH_2$
iii. 8 molecules of NADH.

So, energy yield :

i. 9 Acetyl CoA	= 12 x 9	= 108	ATP
ii. 8 FAD-2H	= 2 x 8	= 16	ATP
iii. 8 NADH	= 3 x 8	= 24	ATP

Total ATP production = 148 (108 + 16 + 24) ATP.

But in the first reaction 2 ATP is required for the initial activation of fatty acid.

So, net gain of stearic acid oxidation = 146 ATP.

Q. ***How many energy (ATP) is formed from 1 molecule of palmitic acid?***

Ans.

Number of acetyl CoA formation from fatty acid :

Formula : $(^{n}/_{2} - 1)$ number of turns

e.g from 16 C palmitic acid 8 acetyl CoA is formed by 7 turns.

One molecule of palmitic contains 16 carbon atoms. In Beta-Oxidation of palmitic acid produces :

i. 8 molecules of acetyl-CoA
ii. 7 molecules of $FADH_2$
iii. 7 molecules of NADH.

So, energy yield :

i. 8 Acetyl CoA	= 12 x 8	= 96	ATP
ii. 7 FAD-2H	= 2 x 7	= 14	ATP
iii. 7 NADH	= 3 x 7	= 21	ATP

Total ATP production = 131 (96 + 14 + 21) ATP.

But in the first reaction 2 ATP is required for the initial activation of fatty acid.

So, net gain of stearic acid oxidation = 129 (131 - 2) ATP.

Total energy production = 129 x 51.6 = 6656 kilo joul of energy

Phospholipids

Chemistry :Phospholipids belong to the group of conjugated fats,containing sugar alcohol or complex amino alcohol, fatty acid, phosphoric acid & nitrogenous base.

Example : Lecithin, cephalin, sphingomyelin etc.

Blood level : Total phospholipid in blood is 215 mg/100 ml.

Classification : Phospholipid may be classified as-

1. *Monoamino-monophospholipid* : 1 molecule of phosphoric acid, 2 molecule of fatty acids, 1 molecule of glycerol & 1 molecule of nitrogenous base.
   Example : Lecithin, cephalin etc.
2. *Diamino-monophospholipid* : 1 molecule of phosphoric acid and 2 molecule of nitrogenous base.
   Example : Sphingomyelin.

Distribution : The phospholipids are widely distributed in the body and are contained within it. They remain in the cell membrane as well as in the protoplasm. Brain, nervous tissue contain the maximum amount of phospholipids.

Functions :

1. Forms the essential constituent of cell wall.
2. Helps in fat digestion & transport.
3. Protect & maintain the permeability of cell.
4. Helps in tissue oxidation.
5. Helps in blood clotting by depressing the fibrinolytic mechanism.

Cholesterol

1. *Chemistry* : It is a complex monohydric secondary alcohol. With fatty acid it form waxes.
2. *Properties* : It is a stable white crystalline substance, insoluble in water but readily soluble in chloroform, ether, alcohol & other fat solvents.
3. *Blood level* : 150-200 mg/100ml of blood.
4. *Distribution* : It present in all cells-both in the cell membrane & cytoplasm. All body fluids contain cholesterol excepting C SF.

Cholesterol turn over : 1 gm/day

i. *Input* : 1 gm/day
   a. Endogenous production : 500 mg/day
   b. Dietary : 500 mg/day
      Food : Egg, milk, liver, brain, skin etc
ii. *Output* : 1 mg/day
   a. Bile salt : 500 mg/day
   b. Faecal neutral steroid : 400 mg/day
   c. Skin desquamation : 100 mg/day

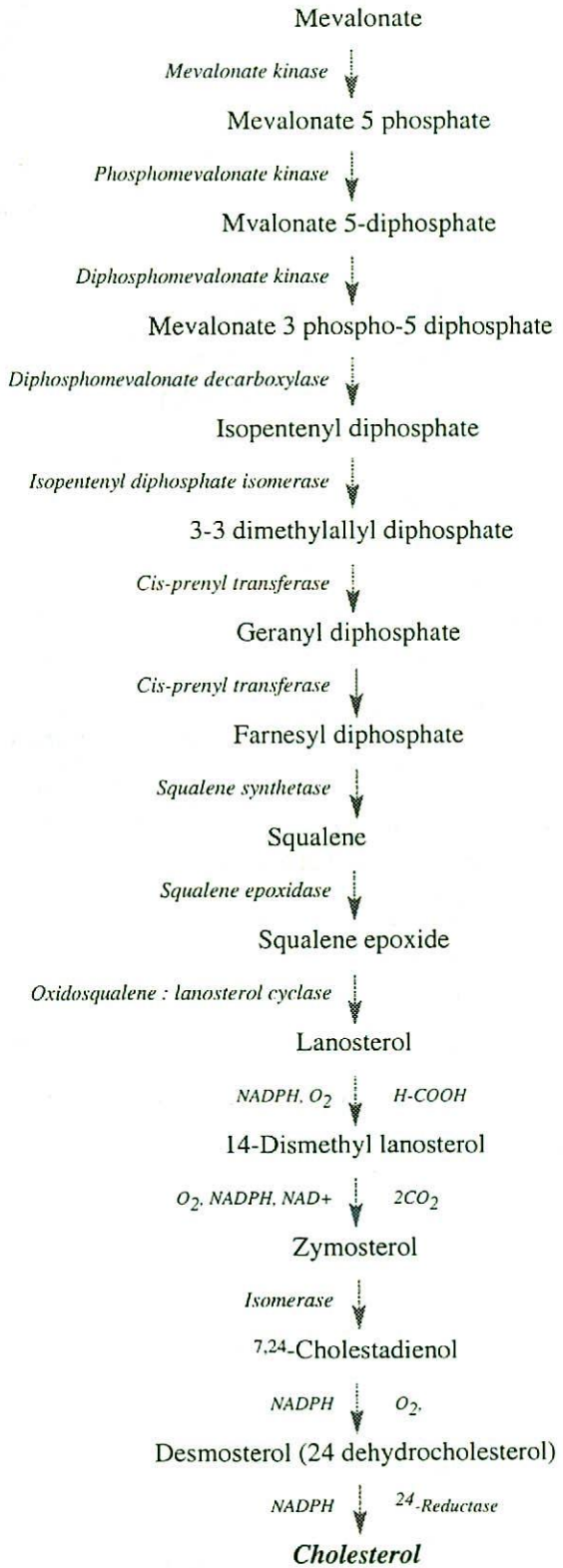
Cholesterol synthesis :

Production site : In steroidogenic organs-

i. Liver
ii. Adrenal cortex
iii. Gonads
iv. Intestine
v. Brain
vi. Skin.

Cholesterol is synthesized in endoplasmic reticulum and cytosol portion of the cell.

Steps of synthesis of cholesterol : The sequence of reactions in the pathway of cholesterol synthesis is given below-

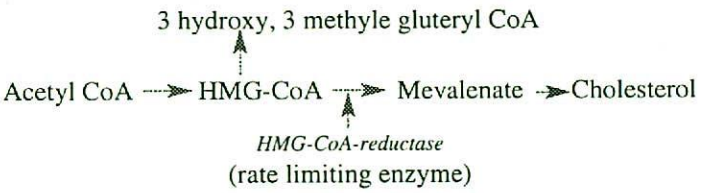


(Ref: Harper's Illustrated Biochemistry 26th Edition, Page-221, 222

1. Activation of acetate to acetyl CoA.
2. Condensation of two acetyl CoA to form acetoacetyl CoA.
3. Formation of 3 hydroxy-3methylglutaryl-CoA (HMG-CoA)
4. Formation of mevalonare.
5. Phosphorylation of mevalonic acid to form 5-phospho mevalonate and 5-diphospho mevalonate
6. The diphosphomevalonic acid after losing CO_2 & H_2O gives rise to form isopentenyl diphosphate.
7. The isopentenyl diphosphate isomerises to form 3-3 dimethylallyl diphosphate which combines with isopentenyl pyrophosphate to give geranyl diphosphate.
8. Another molecule of isopentenyl diphosphate combines with geranyl diphosphate to form farnesyl pyrophosphate.
9. Two molecule of farnesyl pyrophosphate combine to form squalene.
10. An oxidocyclase converts squalene to lanosterol.
11. Lanosterol converts into cholesterol by a number of transformation.

Regulation of cholesterol synthesis :

1. Fasting condition decreases and well fed condition increases cholesterol synthesis.
2. Increase dietary cholesterol decreases cholesterol synthesis and vice versa.
3. Increase insulin and thyroxin decreases cholesterol synthesis.
4. Glucagon and cortisol decreases cholesterol synthesis.
5. Feed back inhibition.



Rate limiting enzyme : Every metabolic pathway is controlled by hormone according to need of the body. This hormones always acts by controlling or stimulating the enzyme in the pathway. This enzyme is called rate limiting enzyme.

* Anti-cholesterol drugs reduce the blood cholestrol by inhibiting rate limiting enzyme HMG-CoA reductase i.e

Increase cholesterol in the cell → Feed back inhibition of HMG-CoA reductase.

Functions of cholesterol :

1. Essential constituent of all cells.
2. Controls cell permeability.
3. Prevents haemolysis.
4. Fat transport.
5. Formation of cholic acid.

6. Antitropic action.
7. Controls cell division.
8. Antagonistic to phospholipids.

Hypercholesterolaemia : Presence of cholesterol in blood above the normal level is called hypercholesterolaemia.

Normal blood level : 150-200 mg/dl of blood.

Relation of cholesterol & atherosclerosis
or coronary heart disease : Cholesterol is the chief pathogenic factor of atherosclerosis. So high cholesterol in food - more chance of atherosclerosis or coronary heart disease.

Chylomicron

Metabolism of chylomicron : By lipoprotein lipase, chylornicrons are metabolized into

i. Chylomicron remnant
ii. Fatty acids: enters into the extrahepatic tissues.
iii. Glycerol
iv. HDL.

Chylomicron remnants are taken up by the liver by receptor-mediated endocytosis. In the liver the cholesterol esters & triglycerides are hydrolyzed into cholesterol & fatty acid.and metabolized.

The LDL (apo B-100, E) receptor and a remnant receptor specific for apo E take part in renmant uptake. Hepatic lipase has a dual role : i. acting as a ligand to the lipoprotein, ii. hydrolizing its TG & phopholipid.

(Ref: Harper's Illustrated Biochemistry 26th Edition)

Keton Body

Keton bodies are :

1. Acetoacetic acid.
2. Beta hydroxy butyric acid.
3. Acetone.

Here acetoacetic acid is called primary keton body.

Normal blood level : 1 mg/100ml of blood.

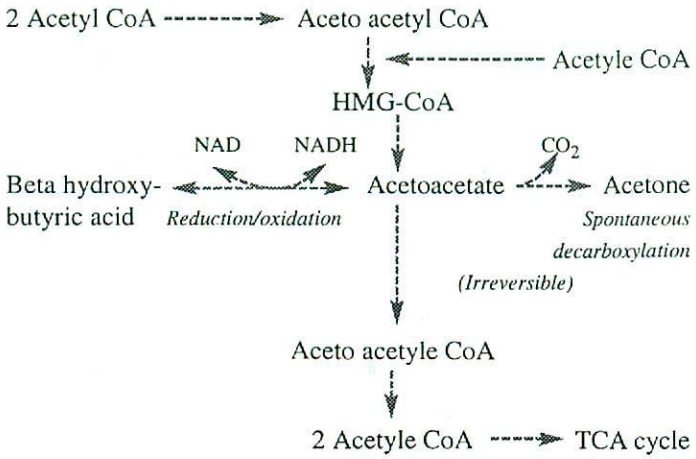
Source of keton bodies : The ketogenic substances are the main source, e.g

i. All the fatty acid.
ii. Ketogenic amino acid, i.e Tyrosine etc.

Site of formation : Liver is the only site.

Mechanism of keton body formation : 2 molecules of acetyl-CoA condense to form acetoacetyl CoA. The reaction is catalyzed by the enzyme beta ketothiolase.
Then by the process of deacylation acetoacetic acid is formed from acetoacetyl CoA. This reaction is catalysed by the enzyme, acetoacetyl CoA deacylase.
When acetoacetic acid is formed, a large portion is converted into beta-hydroxybutyric acid by reduction. This reaction is catalyzed by enzyme beta hydroxybutyrate dehydrogenase.

Small portion of acetoacetic acid is spontaneously converted into acetone by decarboxylation process. It occurs mainly in the lung.



(Ref. Harper 26th edition).

Factors of ketogenesis :

i. Substratre : Acetyle CoA
ii. Product : Ketone body
iii. Site : Only liver
iv. Compartment : Mitochondria

Route of ketone body excretion :

i. Kidney : Beta hydroxybutyric acid, acetoactate
ii. Lungs : Acetone (which is volatile).

Disposal of ketone body :

i. Utilization as metabolic fuel by-
   a. Cardiac muscle
   b. Skeletal muscle
   c. Neurons
ii. Excretion from body.

Most commonly found in blood : Beta hydroxybutyric acid

Ratio : Beta hydroxybutyric acid : Acetone = 3 : 1

Normal ketone body concentration in blood = < 3 mg%
= < 0.2 mmmol/L

Fate of ketone body

I. *Acetoacetic acid* :
   1. It reduces to form beta hydroxy butyric acid.
   2. It decarboxylase to form acetone.
   3. It may reform aceto-acetyl CoA.
   4. It may be used for production of energy.

II. *Beta hydroxybutyric acid* :
   1. It may be converted into acetoacetic acid by reversible reaction.
   2. It is converted into β hydroxy buteryl CoA then aceto-acetyl CoA in the liver.

III. *Acetone :*
1. It may evaporates & passes out through expiration.
2. A small amount is converted into acetoacetic acid.

Acetone :
i. Produced by non enzymatic spontaneous decarboxylation of acetoacetate.
ii. Always excreted through lungs during expiration of air.
iii. Can not be used as metabolic fuel.
iv. Can not cause keto acidosis as it is not an acid.

Antiketogenic substances

These are the substances which prevent the formation of ketone bodies. *They include-*
i. All carbohydrates
ii. Antiketogenic amino acid
ii. Glycerol part of fat.

Some important terms

i. ***Ketonemia*** : Very high concentration of ketone body in blood. Ketonemia is always associated with ketonuria.

ii. ***Ketonuria*** : Increase excretion of ketone body through urine after crossing renal threshold.

iii. ***Ketosis*** : Ketonemia + ketonuria.

Accumulation of abnormal amount of ketone bodies in the tissue and tissue fluids is termed as ketosis, where the urinary excretion of β-hydroxybutyric acid exceeds 200 mg daily (normal 5-10 mg).

Conditions leading to ketosis-
a. Diabetes mellitus.
b. Starvation
c. High fat or low carbohydrate diet.
d. Muscular exercise.

iv. ***Ketoacidosis*** : Ketonemia + ketonuria + ketosis. Acidosis due to keto acids.

Metabolism of ketone bodies :

i. Anabolism : Synthesis of ketone bodies.

ii. Catabolism : After the synthesis in the liver, ketone bodies are transported from the liver and the utilization & oxidation of them occurs in extrahepatic tissues. The main pathway of utilization of ketone bodies in extrahepatic tissues for the activation of acetoacetate to acetoacetyl-CoA involves succinyl-CoA and the enzyme succinyl-CoA-acetoacetate CoA transferase.

Acetoacetate reacts with succinyl CoA, leaving free succinate. Then the acetoacetyl-CoA is splitted to acetyl CoA by thiolase and oxidized in the TCA cycle.

Ketone bodies are oxidized in extrahepatic tissues proportionately to their concentration in the blood.

(Ref Harper's 26th edition)

Biomedical importance of ketone bodies :

1. Ketone bodies are important sources of energy for the peripheral tissues (extrahepatic tissues such as skeletal & cardiac muscle and renal cortex).
2. They are utilized in the brain as fuel during prolonged periods of fasting.
3. In case of starvation & diabetes mellitus, the rate of ketone bodies formation is greater than the rate of their use. So, their concentration begin to rise in the blood (ketonemia) & eventually in the urine (ketonuria).

Ketosis

Definition : Higher than normal quantities of ketone bodies present in the blood or urine constitute ketonemia (hyperketonemia) or ketonuria, respectively. The overall condition is called ketosis.

Causes of ketosis :

I. Physiological cause :
a. Starvation
b. Low carbohydrate diet.
c. High fat diet.

II. Pathological cause :
a. Diabetes mellitus.
b. Pregnancy toxemia.

(Ref Harper's 26th edition)

Production of ketosis in absence of carbohydrate intake :

Absence of carbohydrate intake
↓
Glucose availability in blood becomes less
↓
Liver glycogen is drawn upon in an attempt to maintain the blood glucose
↓
Concentration of insulin in blood decrease & glucagone increase
↓
Glucose utilization in adipose tissue & the inhibitory effect of insulin on lipolysis becomes less
↓
Fat is mobilized as free fatty acids & glycerol
↓
Increase β-oxidation
↓
Increase Acetyl CoA

↓

Increase acetoacetate

↓

Increase Ketone bodies than normal

↓

Ketosis is produced.

Laboratory test of ketone bodies

Normally ketone bodies are absent in urine but in certain conditions (like uncontrolled diabetes mellitus, starvation) are found in urine.

We can detect ketone bodies in laboratory by the Rothera's test.

Method :

i. 3 ml of urine is saturated with $(NH_4)_2SO_4$ taken in a test tube

ii. Add few drops of 5% Na-nitroprusside & shake

iii. Add cone. NH4OH solution by the side of test tube

iv. Purple coloured ring develops at the junction of two liquids

Result : Presence of Ketone bodies in urine.

Ketoacidosis

i. *Definition* : Ketone bodies are acidic and when produced in excess over long periods, causes a condition known as ketoacidosis (as in diabetes mellitus).

ii. *Mechanism of formation of ketoacidosis in diabetes meliitus* : Decrease insulin causes increase lipolysis to liberate fatty acids. Liver uptakes this fatty acids & converted it into acetyl-CoA. Normally acetyl-CoA enters the TCA cycle but in absence of insulin, more acetyl-CoA is formed than can enter the cycle. This excess acetyle-CoA is converted to acetoacetic acid which, later on, converted to other ketone bodies and forms ketoacidosis.

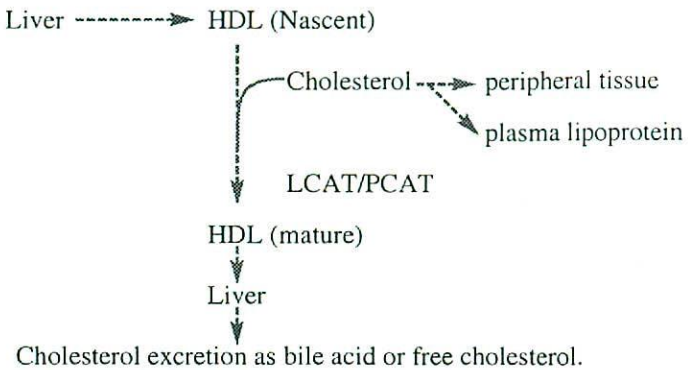
i.e Lack of insulin → Increase catabolism → Increase glycogenolysis, increase gluconeogenesis, increase lipolysis → Increase FFA in plasma → Increase β-oxidation → Increase Acetyl-CoA → Increase Acetoacetate → Increase Ketone bodies → Hyperketonemia → Acidosis → *Diabetic ketoacidosis.*

Physiological basis of treatment of keto acidosis :

Physiological basis of treatment of keto acidosis is to achieve as possible as normal metabolism. Ketoacidosis can be treated by giving-

1. Appropriate insulin
2. Oral hypoglycaemic agents
3. Diet containing
   i. Protein : Anti-ketogenic amino acids (e.g glycine, alanine etc.)
   ii. All carbohydrate
   iii. Fat : Glycerol part.

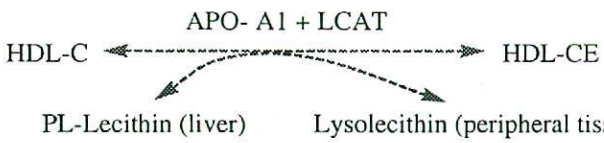
HDL metabolism (scavenger of periferal tissue)



Function of HDL :

i. Pick up cholesterol from peripheral tissue, backs to the liver and facilitate excretion.

ii. *Reverse cholesterol transport* :



Cholesterol after coming incontact with HDL, converted to cholesterol ester and captured by addition of fatty acid phospholipid; converted to lysolecithin.

In liver : Reverse reaction occurs and cholesterol become free from HDL. This is called *reverse transport of cholesterol.*

Importance : Maintenance of normal cholesterol level static.

SDA

The specific dynamic action (SDA) of a food stuff is the extra heat production over and above the caloric value of a given amount of food, which is produced when the food is used by the body.

Specific dynamic action of :

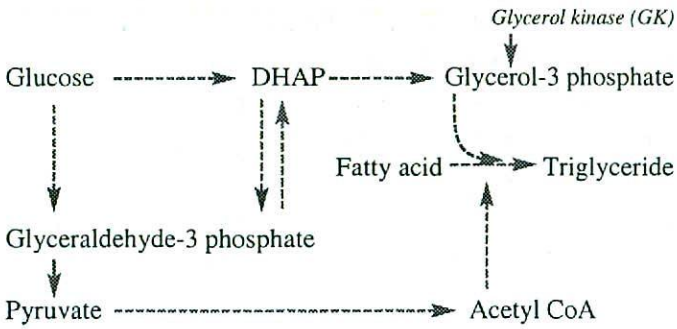
i. Protein : 30%

ii. Fat : 23%

ii. Carbohydrate : 6%

Synthesis of triglyceride (TG) from Glucose :

CHO (carbohydrate) very quickly convert to fat.



Glycerokinase enzyme present only in liver. So fat synthesis occur in liver & adipose tissue. In case of adipose tissue there is no glycerokinase. So fat synthesis occur through formation of pyruvate via EM-pathway. So continuous supply of glucose should ensured i.e dependent on insulin.

Role of insulin :

A. Decrease glucose entry to adipocyte
B. Increase hormone sensitive lipase
↓
Increase breakdown of triglyceride
↓
Increase fatty acid in the liver
↓
Increase fatty acid in the blood.

Hormones of integrated metablism :

i. Insulin
ii. Glucagon
iii. Catecholamines
iv. Cortisol
vi. Growth hormones.

Fuel reserve :

i. Triglycerides : 80 % (1-1.5 lac K.cal)
ii. Glycogen : (1100 K.cal)
iii. Protein : 35000 K.cal).

Fuel interconversion :

Carbohydrate ----------→ Fat
Carbohydrate ←----------→ GAA
Amino acid ----------→ FAT.

Fuel profile :

	Fed *0-4 hours*	*Fasting* *4-24 hours*	*Short starvation* *1-7 days*	*Long starvation* *>7 days*
i. Brain	Glucose	Glucose	Glucose	ketone body
ii. Heart	Fatty acid	Fatty acid	Fatty acid ketone body	Ketone body Fatty acid
iii. Skeletal muscle	Glucose	Fatty acid	Fatty acid Ketone body	Fatty acid

Brain intake

i. Fasting to short starvation : Glucose intake from-
   a. Glycogenolysis
   b. Neoglucogenesis
ii. Long starvation : Ketone body.

Metabolism in well fed condition (2-4 hours after meal)

i. Increase glycogenesis
ii. Increase lipogenesis
iii. Increase protein synthesis
iv. Increase HMP-shunt
v. Increase glycolysis in brain and skeletal muscle.

Metabolism in starvation

Aims :

i. Maximum use of triglycerides
ii. Maintenance of blood glucose concentration
iii. Increase ketone body synthesis
iv. Decrease body catabolism.

In fasting (4-24 hours after meal)

i. ↑↑↑ Glycogenolysis
ii. Lipolysis
iii. Gluconeogenesis
iv. Ketogenesis.

In short starvation (24 hours to 7 days)

i. ↑↑↑ Gluconeogenesis
ii. ↑↑↑ Proteolysis
iii. ↑↑↑ Lipolysis
iv. ↑↑↑ Ketogenesis.

In prolong starvation (>7 days)

i. ↓↓ Gluconeogenesis and proteolysis
ii. ↑↑↑↑ Lipolysis and ketogenesis further increase.

Diabetes

Metabolic changes in type I diabetes

Hyperglycemia and ketoacidasis are the hallmarks of untreated diabetes mellitus.

i. *Hyperglycemia* is caused by increased hepatic production of glucose combined with diminished peripheral utilization.
ii. *Ketosis* results from increased mobilization of fatty acids from adipose tissue combined with accelerated hepatic synthesis of 3-hydroxybutyrate and acetoacetate.
iii. However, not all the fatty acids flooding the liver can be disposed of through oxidation or ketone body synthesis. Fatty acids are also converted to *triacylglycerol*, which is packaged and secreted in *VLDL*.
iv. Chylomicrons are synthesized from dietary lipids by the intestinal mucosal cells following a meal. Because lipoprotein degradation catalyzed by *lipoprotein lipase* in adipose tissue is low in diabetics, the plasma chylomicron and VLDL levels are elevated, resuting in ***hypertiglyceridemia***.

v. These metabolic changes result from a deficiency of *insulin* and a relative excess of *glucagon*, the latter playing a critical role in stimulating *gluconeogenesis* and *ketogenesis*.

(Ref. Lippincott's Illustrated Reviews of Biochemistry, 3rd Edition)

Chronic effects of diabetes

The long-standing elevation of blood glucose is widely believed (but not conclusively proved) to cause the *chronic complications of diabetes-*

i. Premature atherosclerosis
ii. Retinopathy
iii. Nephropathy
iv. Neuropathy.

Intensive treatment with insulin delays the onset and slows the progression of these long-term complications. The benefits of tight control of blood glucose outweigh the increased risk of severe hypoglycemia. How hyperglycemia causes the chonic complications of diabetes is unclear. In cells where entry of glucose is not dependent on insulin, elevated blood glucose leads to increased intracellular glucose and its metabolites. For example, increased intracellular sorbitol may contribute to the formation of cataracts. Further hyperglycemia may promote the condensation of *glucose* (or *its metabolites*, particularly *Glyceraldehyde 3 phoshate*) with cellular *proteins*, in a reaction analogous to the formation of HbA_{1c}. These glycated proteins may mediate some of the early microvascular changes of diabetes.

(Please see page 130 of Lippincott's Illustrated Reviews of Biochemistry for discussion of the sorbitol pathway).

(Ref. Lippincott's Illustrated Reviews of Biochemistry, 3rd Edition)

Comparison of two types of diabetes mellitus

	Insulin-dependent diabetes mellitus (IDDM)	*Non-insulin-dependent diabetes meliitus (NIDDM)*
Synonym	Type I; juvenile-onset diabetes	Type II : adult-onset diabetes
Age of onset	Usually during childhood or puberty	Frequently after age 35
Nutritional status at time of onset of disease	Frequentlv undernourished	Obesity usually present
Prevalence	10%-20% of diagnosed diabetics	80%-90% of diagnosed diabetics
Genetic predisposition	Moderate	Vety strong
Defect or deficiency	β-Cells destroyed, eliminating production of insulin	Inability of β-cells to produce appropriate quantities of insulin : *insulin resistance*
Ketosis	Common	Rare
Plasma insulin	Low to absent	Normal to high
Acute complications	Ketoacidosis	Hyperosmolar coma
Oral hypoglycemic drugs	Unresponsive	Responsive
Treatment with insulin	Always necessary	Usually not required.