ANP 308
METABOLISM OF CARBOHYDRATES, LIPIDS,
PROTEINS AND NUCLEIC ACIDS

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MAIN COURSE

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MODULE 1

UNIT 1   DEFINITION AND CLASSIFICATION OF CARBOHYDRATES

CONTENTS

1.0   INTRODUCTION

Carbohydrates play an important role in the supply of energy, structural rigidity and formation of RNA and DNA in living organisms (plants and animals). Carbohydrates come in different forms (classes) and the ability of carbohydrates to carry out the above mentioned functions depends on the type (class) of carbohydrate.

In livestock nutrition, carbohydrates serve as the major energy sources. However, the ability of livestock to utilise the carbohydrate will depend on the type of carbohydrate and the type of livestock (whether monogastric or ruminant). It is, therefore, imperative for us to study the carbohydrate type available and some of their chemical reactions.

2.0   OBJECTIVES

By the end of this unit, you will be able to:

• explain the different classes of carbohydrate and their properties
• describe the structures of the carbohydrates
• explain that carbohydrates consisting of 10 or more monosaccharides are referred to as polysaccharides
• discuss that starch is a storage carbohydrate found in plants.

3.0   MAIN CONTENT

3.1   Definition of Carbohydrates

Carbohydrates simply put, mean hydrated carbon because many of them can be represented by the simple stoichoimetric formula (CH20)n. This formula is an over-simplification because many carbohydrates (saccharides) are modified, and contain amino, sulphate and phosphate groups. Generally speaking, carbohydrates are a group of organic compounds that include sugars and related compounds. However, chemically, carbohydrates are polyhydroxy aldehydes and ketones, or substances which yield them (aldehydes and ketones) upon hydrolysis. In this respect, the group termed carbohydrates include sugars, starches, cellulose, gums, pectins, saponins, glucosinolates, cyanogenic glucosides, lectins, glycogen, chitin, etc.

3.2   Classification of Carbohydrates

Carbohydrates are classified into three broad groups, namely:

1.   Monosaccharides
2.   Oligosaccharides
3.   Polysaccharides

We will now take each of these carbohydrates (above) and discuss them in details. The type of carbonyl group is denoted by the prefix of aldo- for an aldehyde and keto- for a ketone, e.g. glyceraldehyde is an aldo-triose. The structures of some common monosaccharides are given below:

3.2.1 Monosaccharides

The monosaccharides are also referred to as simple or monomeric sugars. The monosaccharide is the fundamental unit from which all carbohydrates are formed. Monosaccharides are, therefore, the simplest carbohydrates. The monosaccharide can be represented by the empirical

formular $(CH_2O)_n$, when 'n', a whole number, is equal or greater than the value 3. The smallest molecules usually regarded as monosaccharides are the trioses with $n = 3$ (The suffix -ose is commonly used to designate compounds as saccharides). Monosaccharides containing two to 10 carbon atoms have been synthesised, and many occur in nature.

Trioses (C$_3$)
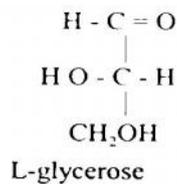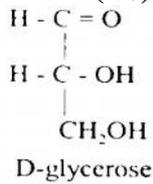
Tetroses (C$_4$)

Pentoses (C$_5$)
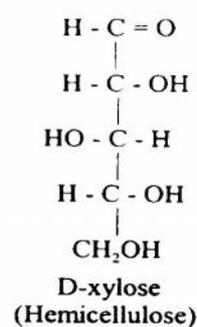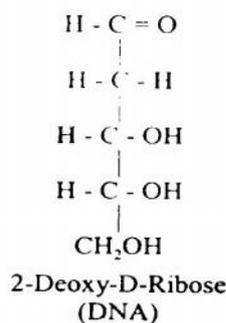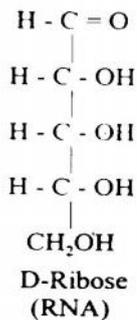
Fig.1.1

Naming of sugars
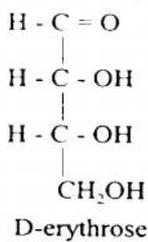The chemical names of sugars and many complex carbohydrates end with the suffix-*ose*. They are also named on a basis of the number of carbon atoms that they contain; tri- for three, and tetra-, petra, hex-, and hept- for 4, 5, 6 and 7, respectively. Note that all the hexoses above are aldehyde except fructose which is a ketone. Because of the presence of asymmetric carbon atoms (labelled with an asterisk) a number of stereoisomers are possible. Some monosaccharides occur in nature while others are synthetic. The hexoses and pentoses are the most important of the simple sugars. The monosaccharides or simple sugars are generally well-crystallined solids, soluble in water, and have more or less sweet taste.

H - C = O
|
HO - C - H
|
HO - C - H
|
HO - C - H
|
CH₂OH

L - Arabinose
(Gums)

CH₂OH
|
C = O
|
HO - C - H
|
H - C - OH
|
CH₂OH

D - Xylulose
(Phosphogluconate pathway)

Hexoses (C₆)

CHO
|
* HCOH
|
* HOCH
|
* HCOH
|
* CHOH
|
CH₂OH

D-glucose

CH₂OH
|
C = O
|
* HOCH
|
* HCOH
|
* HCOH
|
CH₂OH

D-fructose

CHO
|
* HCOH
|
* HOCH
|
* HOCH
|
* HCOH
|
CH₂OH

D-galactose

CHO
|
HOCH
|
HOCH
|
HCOH
|
HCOH
|
CH₂OH

D-mannose
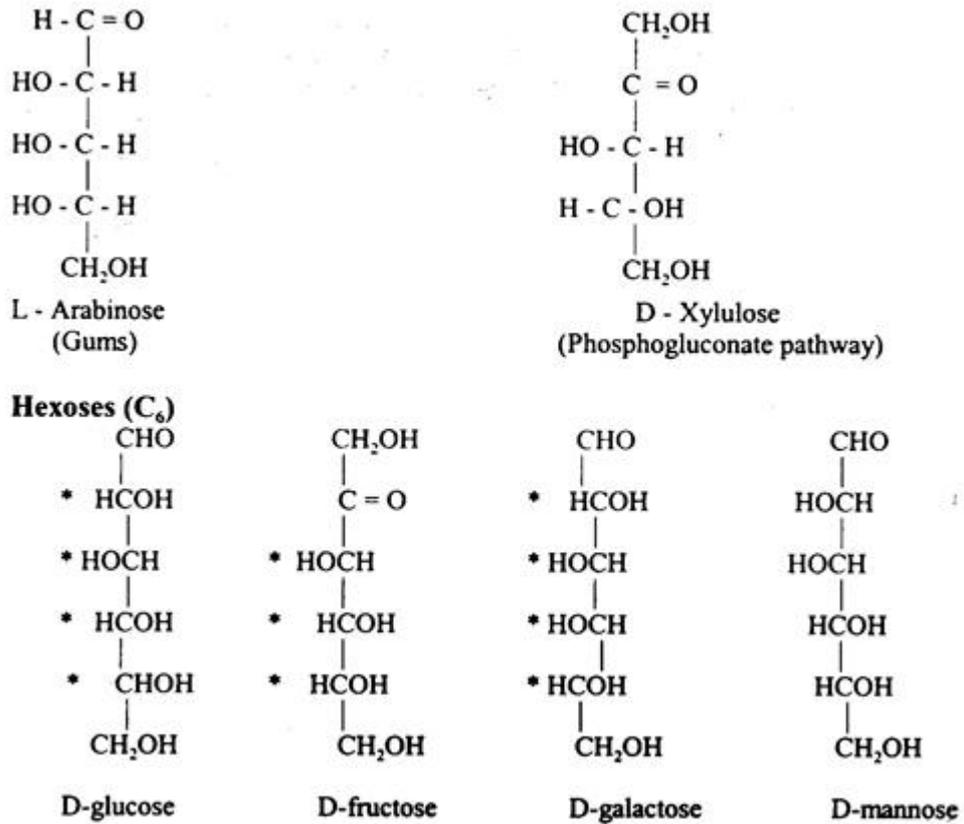
Fig.1.2

Pentoses and hexoses with five and six carbon atoms respectively have the potential to form very stable ring structures via internal hemiacetal formation. The bond angles characteristics of carbon and oxygen bonding are such that rings containing fewer than five atoms are strained to some extent, whereas five- or six-numbered rings are easily formed. In principle, aldotetroses can also form five-numbered ring structure, but they rarely do. Hemiacetals with five-membered rings are called FURANOSES, while those hemiacetals with six-membered rings are called PYRANOSES (Figure 1.3). However, we should note that in cases where either five or six-membered rings are possible, the six-membered ring usually predominates. For example, for glucose less than 0.5% of the furanose forms exist at equilibrium. Why? The reason for this is not yet clear; but furanoses and pyranoses are more realistically represented by pentagons or hexagons as in Haworth Convention. In another way, the structures can also be represented as straight chain showing the acetal bonding as described in the Fisher Projection.

Fructose ($C_6H_{12}O_6$)
(fructofuranose)

β-D-glucose in the pyranose
(glucopyranose)
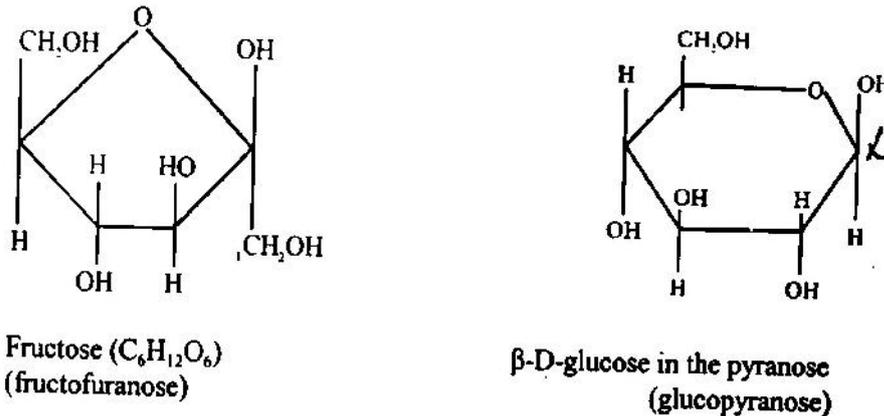
Fig.1.3: Rug Structure of Six Carbon Atom Compounds
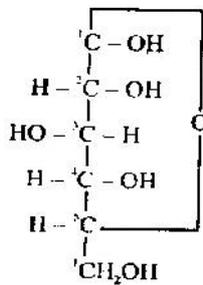
- D - Glucose
Fig.1.4:(Fisher projection)

3.2.2 Disaccharides

Two molecules of simple sugars (monomers) are linked together by an acetal to form a disaccharide. The two simple sugars may either be similar or different. The following features therefore distinguish one disaccharide from another.

a.   the two specific sugars involved and their stereo configuration. (Remember the stereoisomerism discussed in lecture I);
b.   the carbons involved in the linkage. Most common linkages are 1- 1, 1 - 2, 1 - 4 and 1 – 6;
c.   the order (arrangement) of the two monomers; and
d.   the anomeric configuration of the hydroxyl group on carbon 1 of each glucose unit.

The disaccharide with a bond between the 1 carbon of   - glucose and 4-carbon of another   - glucose is called a Maltose. The bond is called   -1, 4 glycosidic link. If, however, the left-hand sugar has been in the   -form before linking, then the compound would be a p-linked disaccharide. The compound of this sort which is comparable to maltose is called a Cellobiose. Lactose, the sugar found in milk, resembles

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cellobiose, but the left-hand sugar is galactose instead of glucose. The structures of some common disaccharides are shown below:

Common disaccharides
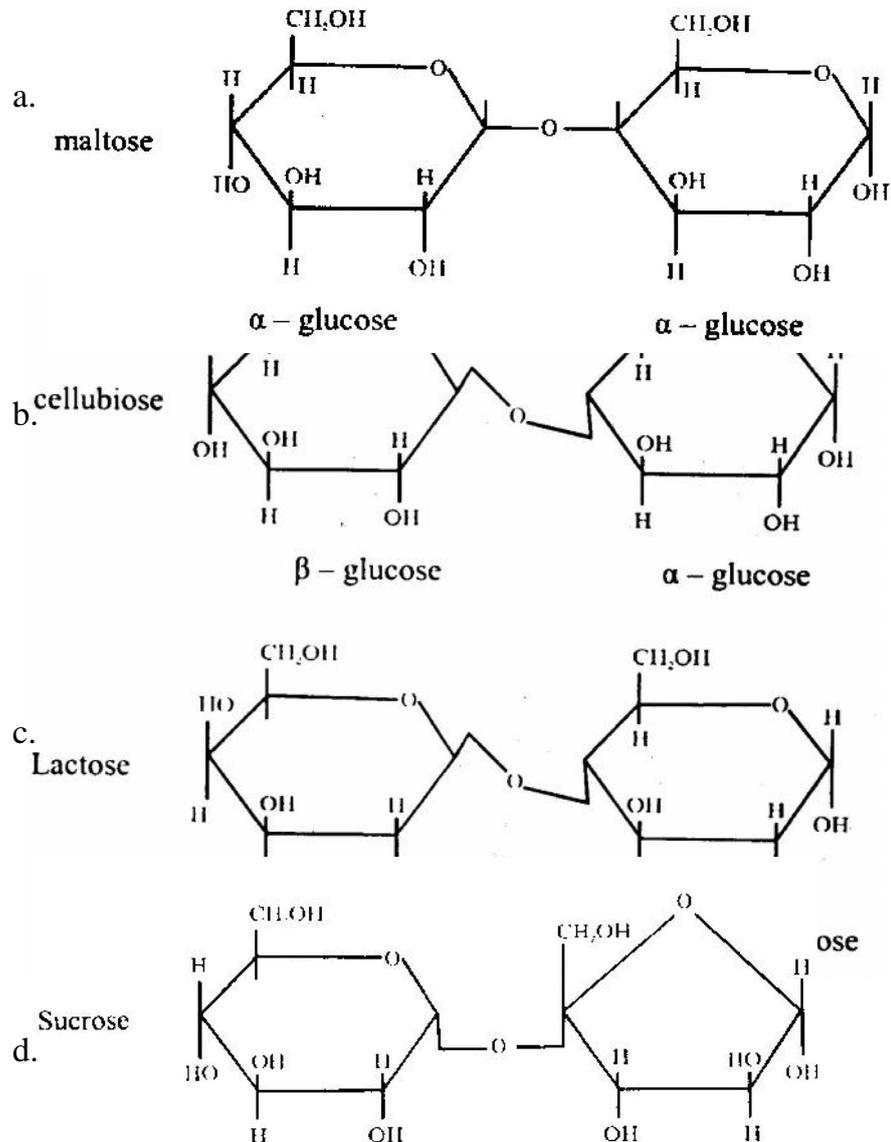
Fig.1.5

Note: with the exception of sucrose, the ring of the right-hand glucose unit can open exposing a free aldehyde group and giving reducing properties to the sugars. Also, the disaccharides are soluble in water, though to varying levels.

Short notes on some disaccharides
i.   Sucrose (Cane Sugar)

Sucrose is made up of a combination of one molecule of D-glucose and one molecule of D-fructose. It occurs in sugar cane; hence, the synonym "cane sugar," and also in beets (major sources of commercial sugar). Sucrose also occurs in ripe fruits,

in tree sap (maple sugar), and in many fruits and vegetables. Sucrose is dextrorotatory, but it is not a reducing sugar as it has no free aldehyde or ketone group. When hydrolysed by dilute acid or the enzyme sucrose, sucrose splits into two constituent's monosaccharides. The resulting sugar is levorotatory. Since the hydrolysis thus results in a change from destrorotation to levorotatum, the process is called inversion and the mixture of glucose and fructose is often termed invert sugar. Such a process is the way by which honey bees convert sucrose of plant nectar to honey.

ii.   Maltose (Malt Sugar)
This disaccharide consists of two molecules of a -0- glucose joined together in an a - I, four linkage. The position of H on the number 1 carbon atom molecule (a) is a position. Note that the number six carbon atoms are in as configuration. Maltose derives its name from the fact that it is produced commercially from starch by the action of malt, obtained from germination barley which contains a starch hydrolysing enzyme distaste.

iii.   Cellobiose
Consist of two molecules of B - D - glucose joined together in a - 1, four linkages. This linkage is the fundamental one for the cellulose molecule and cannot be split by mammalian enzyme. It can be split, however, by microbial and fungal enzymes or acid. Cellobiose does not occur in free form in nature but only as a component of glucose polymers.

iv.   Lactose (Milk Sugar)
This is the sugar of milk and consists of one molecule of   - D-glucose and one molecule of   -D - galactose, joined in a linkage. This linkage can be separated by the enzyme lactase or by the addition of acid. It is a reducing sugar and is only one sixth as sweet as sucrose. Lactose is of special interest in nutrition, because it makes up nearly half of the solids of milk and because it does not occur in nature except as a product of the mammary gland. Having discussed the mono-and disaccharides, we shall now focus on the third and last class of carbohydrates - the polysaccharides

3.2.3 Oligosaccharides

The oligosaccharides contain sugars with two to ten glucose units joined together by glycosidic bonds. The oligosaccharides are therefore formed by the combination (coming together) of two or more (maximum of 10) of the monomers. The monomer sugars may be of same sugars or

different monomer sugars. Examples of some common oligosaccharides are mentioned below:

a.   Disaccharides: made up of two monomer sugars, e.g. sucrose, maltose, cellubiose.
b.   Trisaccharides: made up of three monomer sugars, e.g. raffinose
c.   Tetrasaccharides: made up of four monomer sugars, e.g. stachyose.
d.   Pentasaccharides: made up of five monomer sugars, e.g. verbascose.

The simplest and biologically most important oligosaccharides are the Disaccharides, made up of glycose units, e.g. sucrose, lactose, maltose, cellubiose, gentiobiose. The types of monomer sugars that make up these disaccharidcs are shown in Table 1.1.

Table 1.1: Composition of Some Disaccharide Sugars

Disaccharide	Structure
Sucrose	Glucose - Fructose
Lactose	Galactose - Glucose
Trehalose	Glucose - Glucose
Maltose	Glucose - Glucose
Cellubiose	Glucose - Glucose
Gentiobiose	Glucose - Glucose

A look at Table 1.1 shows that glucose appeared as a constituent of all the disaccharide. This underscores the importance of glucose as a substrate in the nutrition of plants and animals. Secondly, a look at maltose, cellubiose and gentiobiose showed that these disaccharides contain only glucose units. The question now is that how can two glucose units combine to give three different products. This may appear confusing at first. A little explanation is therefore needed at this stage. This will depend firstly on whether the connecting sugars are   or   type and secondly, it will also depend on the points at which the sugars are connected to each other. These points are illustrated by discussing how the disaccharides are formed from two units of monomers.

The disaccharides derive their name from the fact that they are a combination of two molecules of monosaccharides. Their general formular, $C_{12}H_{22}D_{11}$ indicates that one molecule of water has been eliminated as two monosaccharides combine

$$C_6H_{12}O_6 + C_6H_{12}O_6 \xrightarrow{\quad H_2O \quad} C_{12}H_{22}O_{11}$$

3.2.4 Polysaccharides

The carbohydrates consisting of 10 or more monosaccharides are referred to as pol-ysaccharides. They may be considered as condensation of polymers in which the monosaccharides (or their derivatives such as amino sugars and uronic acids) are joined together by glycosidic (acetal) linkages. Polysaccharides are also called glycans and they consist of two types namely homogl ycans and heteroglycans. Homoglycans are polysaccharides that consist of a single kind of monosaccharide, while heteroglycans consist of more than one kind of monosaccharide. Polysaccharides consisting mainly of glucose are called glucans; while those consisting of fructose, mannose and xylose alone are referred to as fructans. mannans and xylans, respectively.

Examples of homoglycans are starches, cellulose, glycogen, insulin, chitin, etc., while examples of heteroglycans are gum acacia, pectins, alginic acids, mucopolysaccharides (hyaluronic acid, heparin, chondroitin sulphates). Generally speaking, polysaccharides are insoluble in water, but upon hydrolysis by acids or enzymes, they are broken down into various intermediate products and finally their constituent monosaccharide units. In this aspect of the course, we shall be concerned with starch, cellulose and glycogen. Other polysaccharides will be discussed in future.

3.2.4.1 Starch

Starch is a storage carbohydrate found in plants. It consists of glucose units. It is therefore a homoglucan (remember our earlier discussion on homoglycans). Starch consists of a mixture of two different types of molecules, amylose and amylopectin. Amylose consists of a long chain of glucose units joined by a-I, 4 linkages while amylopectin consists of a mixture of -1, 4 links with occasional - 1, 6 branches (Figure 1.6). The branches occur after about 25 straight a- 1, 4 bonds. Starches from different sources vary in the ratio of amylose and amylopectin, in the size of the individual molecules, in general amylopectin accounts for about 70 per cent of starch.
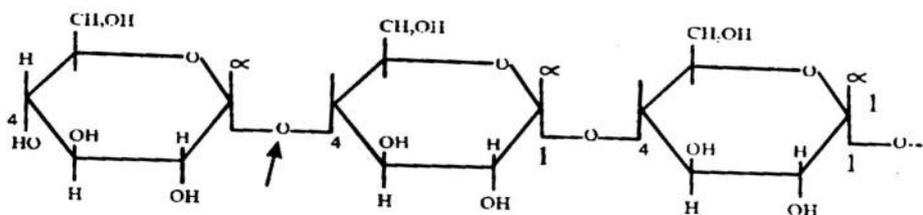

Fig. 1.6: Structure of Amylase

The structure above is the glucose units of amylose linked in an unbranched chain. The amylose structure can therefore be considered as

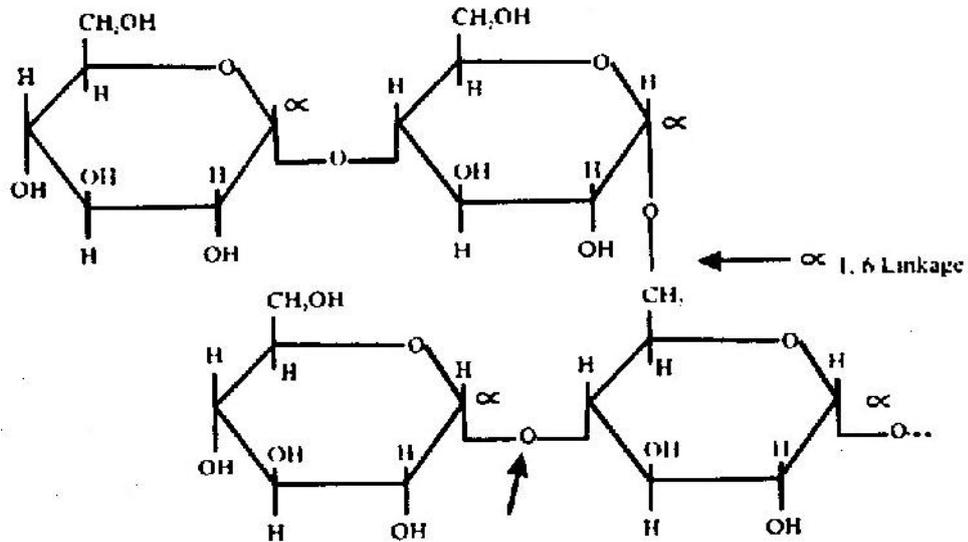an expanded maltose structure with a free sugar group on one end

Fig.1.7: Structure of Amylopectin

Amylopectin also contains chains of glucose units like those of amylose, also has branches of these glucose chains linked through the 6 - OH of glucose in the manner as shown in Figure 1.7. The long chains of amylose roll themselves into a stable helix shape which is held in place by hydrogen bonding. The helix is a tube into which other molecules or atoms can fit. One example of this is the fact that iodine can fit inside the helix and form a blue coloured complex with amylose, a reaction which is often used to detect the presence of starch or iodine. The bluer the colour obtained, the more the amount of amylose component of the starch. Amylose is soluble in hot water while amylopectin is insoluble in hot water. Starches from different plants when viewed microscopically show difference in shapes and sizes (appearances). This property furnishes the basis for microscopic identification of different types of starches.

Some starches show a high degree of hydrogen bonding and such starches are quite resistant to rupture. Tuber starch, such as found in the potato, is extremely resistant and must be cooked before being utilised by species such as pigs or chickens. Starch type in plants is genetically determined. However, starch modification techniques are available and have applications in the food industry. Dextrin is an intermediate resulting from the hydrolysis and digestion of starch as well as the action of heat on starch.

3.2.3.2 Cellulose

This is the most abundant substance in the plant kingdom and is a major structural component of plant cell walls. Cellulose is made up of polymerised glucose molecules ranging from 900 - 2,000 molecules. Cellulose is also a glucan. Chemically, cellulose is a polymer of - 1,4 - linked D - glucose units. As such, the six carbon atoms are in the transposition which results in cellulose being flat, band-like microfibril. Natural cotton is one of the purest forms of cellulose. Cellulose is not subject to attack by the digestive enzymes of man and other monogastrics, hence it is an important source of bulk in the diets. Contrarily, microbes in the rumen of ruminants can secrete cellulose enzyme which can degrade cellulose. Cellulose is not soluble in water but soluble in ammonical solution of cupric hydroxide, HCl acid solution of zinc chloride.

3.2.3.3 Glycogen

This is the storage form of carbohydrates in animals and fungal cells. Glycogen is deposited in the liver, which acts as a central energy storage organ in many animals. Glycogen is also abundant in muscle tissue, where it is more immediately available for energy release. The structure of glycogen is of D-glucose combined with - 1, 4 linkage and an - I, 6 cross linkage, very similar to that of amylopectin (component of starch moeity), except that the molecules are larger and the cross linkages move frequently (once every 15 or so straight bonds). Glycogen gives a red-brown, red, or at times, violet colour with iodine and which yields D - glucose upon complete hydrolysis.

4.0   CONCLUSION

Carbohydrates make up most of the organic structures of some plants and some animals and are produced by the process of photosynthesis.

5.0   SUMMARY

Carbohydrates are classified into three major groups, namely-monosaccharides, oligosaccharides and polysaccharides, respectively. The monosaccharides are the simplest forms of sugars and make up oligosaccharides and the polysaccharides. The carbohydrates can be represented by chemical and structural formulae. The structural formulae are either represented in straight chain or in ring forms. This is specially represented by the hexoses (6 - carbon sugars).

6.0   TUTOR-MARKED ASSIGNMENT

1. a.   What are monosaccharides, oligosaccharides and polysaccharides?
   b.   Using necessary chemical structures, give two examples of the classes of carbohydrates listed in 1 (a) above.
2.   Write short notes on the following:
        a.   Glycans
        b.   Starch
        c.   Cellulose.

7.0   REFERENCE/FURTHER READING

Maynard, L.A., Lopsli, J.K., Hintz, H.F. & Warner, R.G. (1983). *Animal Nutrition*. (7th ed.). Tata McGraw Hill Publishing Company Limited.

UNIT 2   METABOLISM OF CARBOHYDRATES

CONTENTS

1.0   INTRODUCTION

The carbohydrates are source of energy for animal nutrition. The monosaccharides and oligosaccharides are efficiently metabolised by simple stomach animals. On the other hand, ruminants contain microbes, which secrete enzymes capable of degrading cellulose. Glycogen is a polysaccharide found in animal and fungal cells. Glycogen is a storage form of carbohydrate and is readily utilised when there is deficiency of energy.

2.0   OBJECTIVES

By the end of this unit, you will be able to**:**

- explain the metabolism of carbohydrates in terms of their digestion, absorption and transport in the tissues
- identify the metabolic pathways of carbohydrate, use and storage of glycogenesis and glycogenolosis.

3.0   MAIN CONTENT

3.1   Digestion

The dietary carbohydrates that are most important nutritionally are polysaccharides and disaccharides, since free monosaccharides are not commonly present in the diet in significant quantities. There is, however, some free glucose and fructose in honey, in certain fruits, and in the carbohydrates that are added to processed foods. The cellular use of carbohydrates depends on their absorption from the Gastrointestinal (GI) tract into the blood stream, a process normally restricted to monosaccharides. Therefore, polysaccharides and disaccharides must be hydrolysed to their constituent monosaccharide units. The hydrolytic enzymes involved are collectively called glycosidases, or, alternatively, carbohydrases.

3.1.1 Disaccharides

Virtually no digestion of disaccharides or small oligo saccharides occurs in the mouth or stomach. In the human it takes place entirely in the upper small intestine. Unlike amylase, disaccharidase activity is associated with the mucosal cells of the microvilli or brush border rather than with the intestinal lumen. Among the types of enzyme activities located in the mucosal cells are lactase, invertase (sucrase), and isomaltase. The latter is not a disaccharidase but instead hydrolyses branched dextrins, as mentioned in an earlier section. Lactase catalyses the cleavage of lactose to equimolar amounts of galactose and glucose, and sucrase hydrolyses sucrose to yield glucose and one fructose residue; sucrase also hydrolyses maltose and maltotriose to free glucose.

3.1.2 Polysaccharides

The glycosidase, a-amylase, assumes a particularly important role in polysaccharide digestion because of its specific hydrolytic action on the -1,4 bonds of the starches. Resistant to the action of this enzyme, therefore, are the -1,4 bonds of cellulose and the   -1,6 linkages that form branch points in the starch amylopectin. The a-amylase hydrolyses the unbranched amylose rapidly into units of the disaccharide maltose and into the trisaccharide malltotriose, the latter subsequently undergoing slower hydrolysis to maltose and glucose. The enzyme's hydrolytic action on amylopectin produces, in addition to glucose, maltose, and maltotriose, a mixture of branched oligo saccharides, or dextrins, the smallest of which are tetrasaccharides and pentasaccharides. Together with the complementary activity of another glycosidase, -dextrinase, which hydrolyses the -1, 6 bonds at the branches, the dextrins are consequently hydrolysed to free glucose.

Digestion of starches actually begins in the mouth, since amylase activity is found in saliva.

But considering the short period of time that food is in the mouth prior to being swallowed, this phase of digestion is of little consequence. However, the salivary amylase action continues in the stomach until the gastric acid penetrates the food bolus and lowers the pH sufficiently to inactivate the activity of the enzyme. Starch digestion is resumed in the small intestine, where amylase of pancreatic origin is secreted into the duodenal contents. Here, the presence of the bile, made alkaline by pancreatic bicarbonate, makes the pH favourable for enzymatic function, and most of the starch digested is through the action of the pancreatic enzyme.

3.2   Absorption and Transport

The wall of the small intestine is comprised of absorptive mucosal cells and mucous-secreting goblet cells that line projections, called villi that extend into the lumen. The absorptive cells have a hairy, projection like surface on the lumen side called microvilli, or brush border. A square millimeter of cell surface is believed to have as many as 2×10^5 microvilli projections. The anatomic advantage of the villi/microvilli structure, as it relates to the absorption of nutrients is that it presents an enormous surface area to the intestinal contents. It has been estimated that the absorptive capacity of the human intestine amounts to about 5,400 g/d for glucose and 4,800 g/d for fructose, a capability that is, of course, never challenged in a normal diet.
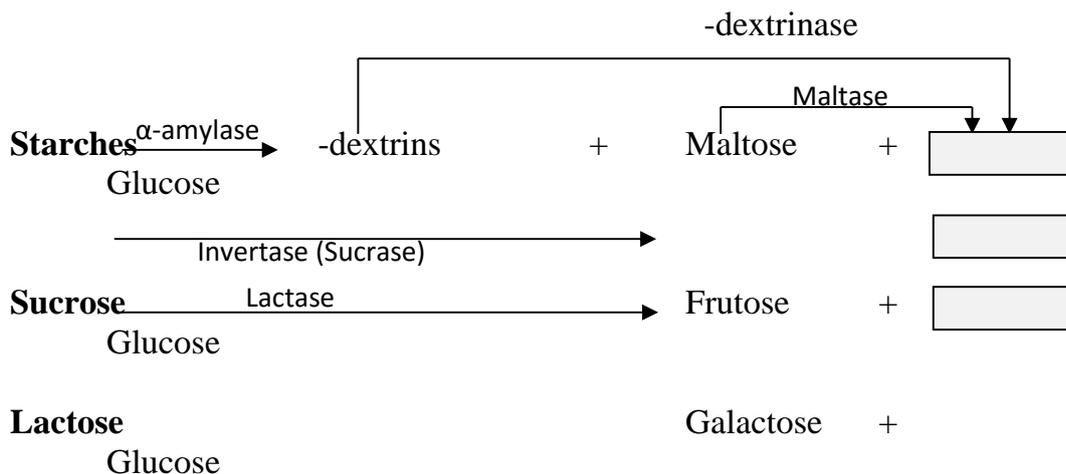

Fig.2.1:The   enzymatic   hydrolysis   of   dietary   carbohydrates, illustrating the importance of glucose as a component of these major nutrients

Glucose and galactose are absorbed across the gut wall by active transport whereas fructose moves across by facilitated diffusion.[1,2] Active transport implies that the process is energy requiring and that a specific receptor is involved. The exact nature of the glucose/galactose carrier is unclear, but is known to be a protein complex connected to the Na^+/K^+ - ATPase pump (p. 16), which, at the expense of ATP, furnishes energy for the transport of sugar through the mucosal cell. Glucose or galactose cannot attach to the carrier until it has been preloaded with Na^+.

Glucose appears to exit the mucosal cell by three different routes: approximately 15 per cent leaks back across the brush border into the intestinal lumen, about 25 per cent diffuses through the basolateral memmbrane; but the major portion (approximately 60 per cent) leaves via a carrier in the serosal membrane. If mucosal cells are poisoned with chemical blockers of oxidative phosphorylation, the transport of glucose and galactose ceases. The fact that some cases of glucose mal-absorption have been attributed to reduced numbers of specific carriers testifies to the importance of this transport mechanism.

Facilitated diffusion, the process by which fructose crosses the mucosal cells, is not energy requiring and can only proceed down a concentration gradient. There is a carrier involved, however, and so the system is saturable and can be competitively inhibited. Since fructose is very efficiently trapped and phosphorylated by the liver, there is virtually no circulating fructose in the bloodstream. Therefore, the downhill concentration gradient for fructose across the intestinal mucosa is ensured.

Following transport across the gut wall, the monosaccharides enter the portal circulation and distribute among various tissues in the body. Gallactose and fructose are readily taken up by liver cells via specific hepatocyte receptors and are subsequently metabolised. Both can be converted to glucose derivatives through pathways that will be discussed later and then stored as liver glycogen or catabolised for energy according to the body's energy demand. The blood levels of galactose and fructose are not directly subject to the strict horrmonal regulation, which is such an important part of glucose homeostasis. However, if they represent a significantly higher than normal percentage of dietary carbohydrate, they may be indirectly regulated hormonally as glucose due to their metabolic conversion to that sugar.

Glucose is nutritionally the most important monosaccharide, since it is the exclusive constituent of the starches and since it also occurs in each of three major disaccharides. Following its active transport through the intestinal mucosal cells it is distributed via the blood stream among

various tissues, primarily liver, muscle, and adipose tissue. It enters
these cells by facilitated diffusion. In skeletal muscle and adipose tissue,
the process is insulin dependent, while in the liver it is insulin
independent. The maintenance of normal blood-glucose concentration is
the net effect of metabolic processes that remove glucose from the blood
for either glycogen synthesis or for energy production and of processes
that return glucose to the blood, such as glycogenolysis and
gluconeogenesis. These pathways, which will be examined in detail in
the next section, are hormonally influenced primarily by the antagonistic
pancreatic hormones insulin and glucagon and the glucocorticoid
hormones of the adrenal cortex.

A rise in blood glucose, for example, following the ingestion of
carbohydrate triggers the release of insulin while reducing the secretion
of glucagon. This results in an increasing uptake of glucose by muscle
and adipose tissue, resulting in the return to homeostatic levels of blood
glucose. A fall in blood glucose concentration conversely signals the
reversal of the hormonal secretions-decreased insulin and increased
glucagon release. Additionally, an increase in glucocorticoid hormone
production occurs in answer to a falling blood glucose level, resulting in
the potentiation of gluconeogenesis, a process to be described in the
following sections.

3.3   Integrated Metabolism in Tissues

The metabolic fate of the monosaccharides depends to a great extent on
the energy demands of the body. According to these demands, the
activity of certain metabolic pathways may be stimulated, while others
may be repressed. The major mediators of this regulation are hormones
such as insulin, glucagon, and the glucocorticosteroids, which activate
or inhibit specific enzymes within the pathways, and allosteric enzymes,
which are stimulated or repressed by certain compounds formed within
the pathway in which the enzymes function. An allosteric, or regulatory,
enzyme is said to be positively or negatively modulated by a substance
(modulator) according to whether the effect is stimulatory or repressive,
respectively.

ATP and its dephosphorylated product, AMP, formed from ADP by
adenylate kinase (2 ADP   AMP + ATP) can modulate certain
allosteric enzymes through opposing effects. This exemplifies the link
between energy demand and allosteric enzyme regulation. As ATP
accumulates, for example during a period of muscular rest, it can
negatively modulate certain regulatory enzymes in energy-producing
pathways so as to reduce the production of additional ATP. An increase
in AMP concentration conversely signifies a depletion of ATP and the
need to produce more of this energy source. In such a case AMP can act

as a positive modulator on regulatory enzymes. The enzyme, phosphofructokinase, which catalyses a reaction in the glycolytic pathway, is modulated by both ATP and AMP in the manner described. The ratio of NADH to NAD also has an important regulatory effect. Certain allosteric enzymes, for example, are responsive to an increased level of NADH, which therefore regulates its formation through negative modulation. Furthermore, dehydrogenase reactions, which involve the interaction of the reduced and oxidised forms of the cosubstrate, are reversible. If metabolic conditions lead to the accumulation of one form or the other, the equilibrium is shifted so as to consume more of the predominant form. The purpose of regulation is to both maintain homeostasis and to alter the reactions of metabolism in such a way as to meet the nutritional/biochemical demands of the body.

The metabolic pathways of carbohydrate use and storage consist of glycogenesis, glycogenolosis, glycolysis and hexose monophosphate shunt, the citric acid cycle, and gluconeogenesis. An integrated overview of these pathways is illustrated and a detailed review of their intermediary metabolites, sites of regulation, and, most importantly, their function in the overall scheme of things will now be considered. Reactions within the pathways will be numbered to allow elaboration of those that are felt to be particularly significant from a nutritional standpoint. Because of the central role of glucose in carbohydrate nutrition, its metabolic fate will be featured.
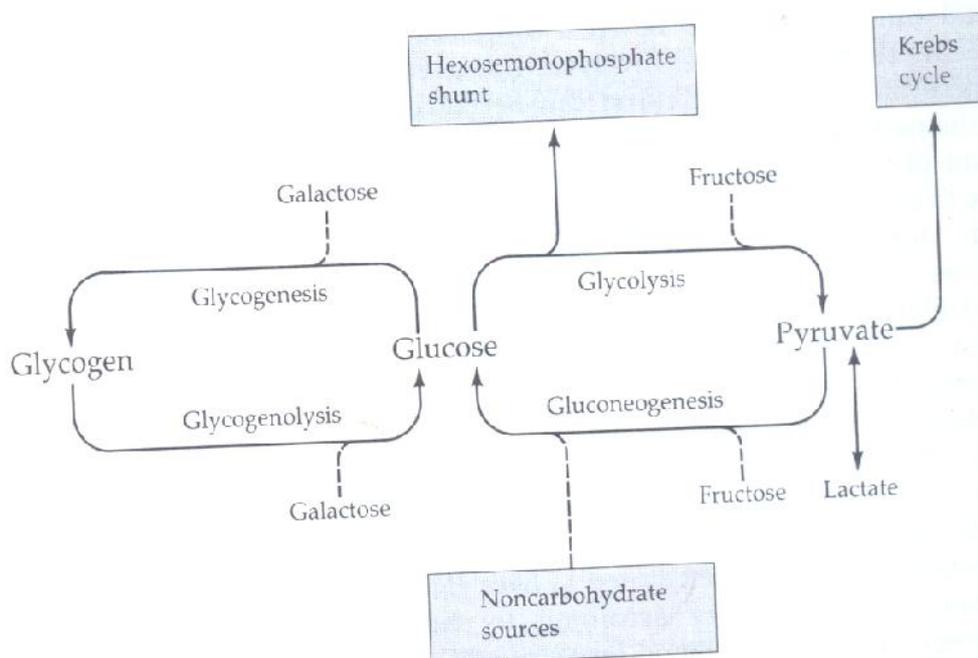

Fig.2.2:An overview of the major pathways of carbohydrate metabolism, emphasising the fate of glucose but also indicating the sites of entry of galactose and fructose into the pathways

3.4   Glycogenesis

Glycogenesis refers to the pathway by which glucose is ultimately converted into glycogen. This pathway is particularly important in hepatocytes because the liver is the major site of glycogen storage. Glycogen accounts for as much as seven per cent of the wet weight of this organ.

The other major site of glycogen storage is skeletal muscle and, to a lesser extent, adipose tissue. It is the glycogen stores that are used first when the body is confronted by an energy demand such as physical exertion or emotional stress, and so the glycogenic pathway is of vital importance in ensuring a reserve of instant energy. The following are comments on selected reactions:

1.   Upon entering the cell, glucose is first phosphoorylated by ATP, producing a phosphate ester at the number 6 carbon of the glucose. In muscle cells the enzyme catalysing this phosphate transfer is hexookinase, an allosteric enzyme that is negatively modulated by the product of the reaction, glucose 6-phosphate. Glucose phosphorylation in the liver is catalysed by glucokinase, and although the reaction product, glucose 6-phosphate, is the same, interesting differences distinguish it from hexokinase. For example, glucokinase is not inhibited by glucose 6-phosphate.

   Also, it has a much higher K_m than hexokinase, meaning that it can convert glucose to its phosphate form even when the cellular concentration of glucose is raised significantly, (e.g, after a carbohydrate-rich meal). The much lower K_m of hexokinase indicates that it is catalysing at maximum velocity even at average glucose concentrations. Therefore the liver has the capacity to reduce blood glucose concentration when it becomes high, and it is noteworthy that glucokinase is deficient in the disease diabetes mellitus. The hexokinase/glucokinase reaction is energy consuming, since the glucose was activated (phosphorylated) at the expense of ATP.
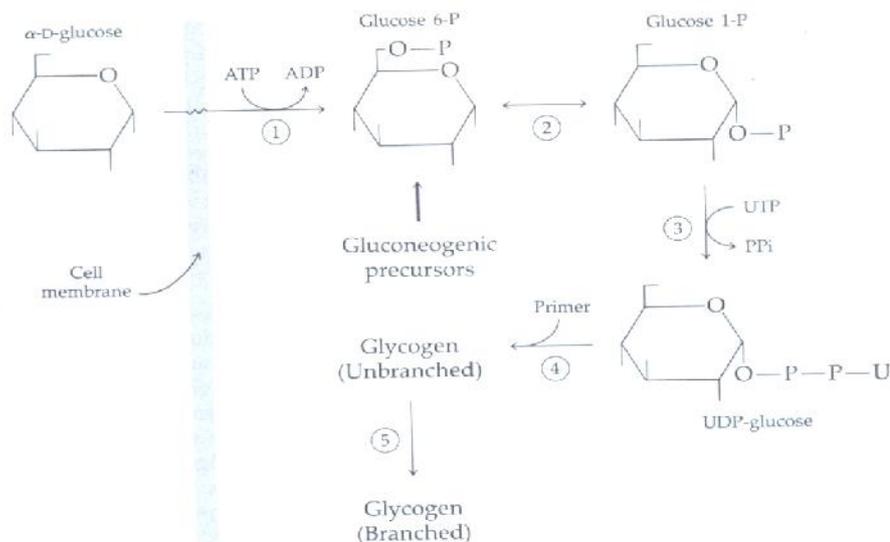
Fig.2.3:Reactions of glycogenesis, by which the formation of glycogen from glucose occurs, glycogen appears to be formed principally from gluconeogenic precursor substances rather than from glucose directly.

2. The phosphate is transferred from the number 6 carbon of the glucose to the number 1 carbon in a complex reaction catalysed by the enzyme phosphoglucomutase.

3. Nucleoside triphosphates sometimes function as activating substances in intermediary metabolism. In this reaction, energy derived from the hydrolysis of the - phosphate anhydride bond of uridine triphosphate allows the coupling of the resulting uridine monophosphate to the glucose I-phosphate to form uridine diphosphate glucose (UDP-glucose).

4. As UDP glucose, the glucose moiety can be incorporated directly into glycogen. The reaction is catalysed by glycogen synthase, and it requires some preformed glycogen (primer) to which the incoming glucose units can be attached. The reaction is stimulated by insulin.

5. Branching within the glycogen molecule is very important because it increases its solubility and compactness and also makes available many non-reducing ends of chains from which glucose residues can be cleaved and used for energy. Glycogen synthase cannot form the -1, Six bonds of the branched points. This is left to the action of the branching enzyme, which transfers small oligosacccharide segments from the end of the main glycogen chain to carbon number six hydroxyl groups throughout the chain. The overall pathway of glycogenesis, like most synthetic pathways, consumes energy, since an ATP (reaction (1)) and a UTP (reaction (3)) are consumed.

3.5   Glenycogolysis

The potential energy of glycogen is contained within the glucose residues that comprise its structure. As the body's energy demand dictates, the residues can be systematically cleaved one at a time from the ends of the glycogen branches and routed through energy-producing pathways. The breakdown of glycogen into individual glucose units, in the form of glucose 1-phosphate, is, called glycogenolysis. Like its counterpart, glycogenesis, it is regulated by hormones, most importantly by glucagon, of pancreatic origin, and the catecholamine hormone epinephrine, originating in the adrenal medulla. Both of these hormones exert positive modulation of the process and are directed at the initial reaction glycogen phosphorylase. They therefore function antagonistically to insulin in regulating the balance between free and stored glucose. The following are comments on selected reactions:

1.   The sequential release of individual glucose units from glycogen is a phosphorolysis process by which the glycosidic bonds are cleaved by phosphate addition. The products of the reaction are glucose 11phosphate and the remainder of the intact glycogen chain minus the one glucose residue. The reaction is catalysed by glycogen phosphorylase, an important site of metabolic regulation by both hormonal and allosteric enzyme modulation. Different forms of glycogen phosphorylase exist, including phosphorylase a, a phosphorylated active form, and phosphorylase b, an unphosphorylated inactive form. The two forms are interconvertible by protein phosphatase, which dephosphorylates phosphoryllase a to its inactive, b form, and by phosphorylase b kinase, which returns the b form to the active, a form. The rate of glycogen breakdown to glucose 11phosphate therefore depends on the relative activity of these enzymes.
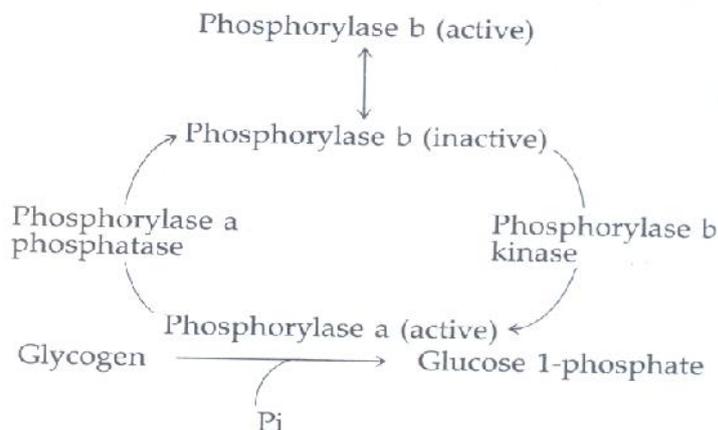

Fig.2.4

The regulation of phosphorylase a phosphatase and phosphorylase b kinase is quite complex. It may involve allosteric modulation by AMP, ATP, glucose 6-phosphate, and Ca^{+2}, and also hormonal regulation by epinephrine (in muscle) and glucagon (in liver), mediated through cAMP. The inter-conversion of active and inactive forms of phosphorylase b, is also allosterically modulated. The textbook by Stryer includes a more in-depth account of the regulation of the phophorylase reaction and its control.

2.   At times of heightened glycogenolytic activity, the formation of increased amounts of glucose 1-phosphate shifts the equilibrium of the glucose phosphate isomerase reaction toward production of the 6-phosphate isomer.

3.   The conversion of glucose 6-phosphate to free glucose requires the action of glucose *6tase*. This enzyme functions in liver and kidney cells but not muscle cells or adipocytes. Therefore, free glucose can be formed from liver glycogen and transported via the blood stream to other tissues for oxidation. It follows that although muscle and adipose tissue have stores of glycogen, these stores can only be broken down for use in these locations. In these tissues the options available to glucose 6-phosphate are formation of glycogen or passage through the glycolytic pathway, which will be discussed next, but not hydrolysis to free glucose. The liver is endowed with all three options.
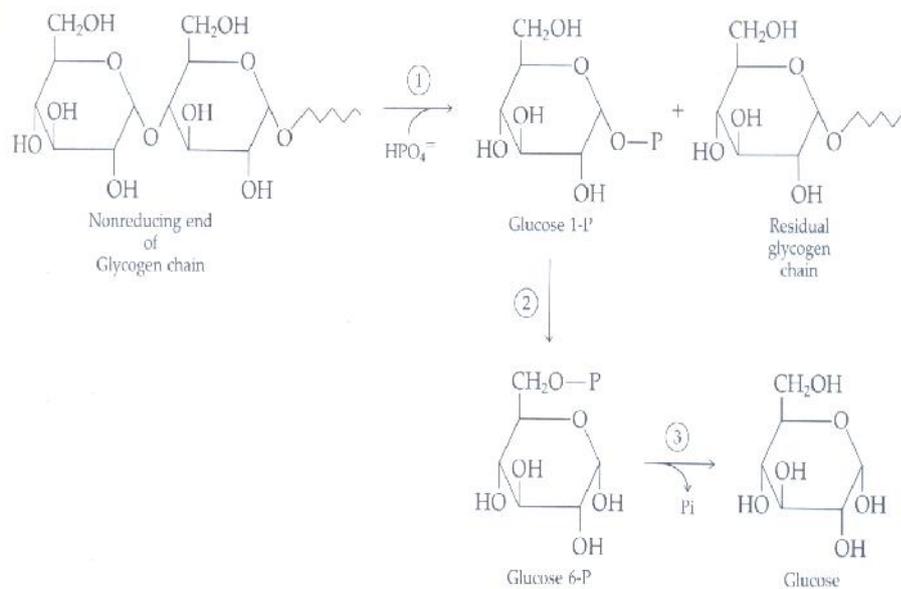

Fig.2.5:The reactions of glyocogenolysis, by which glucose residues are sequentially removed from the non-reducing ends of glycogen segments.

3.6   Glycolysis

Glycolysis is, by definition, the pathway by which glucose is converted into two units of lactic acid, a triose. The pathway can function anaerobically, and in situations in which oxygen debt is in effect, as in times of strenuous exercise, lactate accumulates in the muscle cells, causing the aches and pains associated with overexertion. The importance of glycolysis in energy metabolism is that it provides the initial sequence of reactions necessary for glucose to be oxidised completely to CO_2 and H_2O via the citric acid cycle. In cells that lack mitochondria, such as the erythrocyte, the pathway of glycolysis is the sole provider of ATP by substrate level phosphorylation of ADP. The glycolytic enzymes function within the cytoplasmic matrix of the cell, while the enzymes catalysing the citric acid (Krebs) cycle reactions are located within the mitochondrion (pp. 8, 9). Further metabolism of the products of glycolysis in the Krebs cycle allows complete oxidation of glucose to CO_2 and H_2O, with maximal energy production. Some of the energy liberated is salvaged as ATP, while the remainder maintains body temperature. Many cell types are involved in glycolysis, but most of the energy derived from carbohydrates originates in liver, muscle, and adipose tissue.

The pathway of glycolysis, showing the entry of dietary fructose and galactose,the following are comments on selected reactions:

1.   The hexokinase/glucokinase reaction consumes 1mol ATP/mol glucose. Hexokinase (not glucokinase) is negatively regulated by the product of the reaction, glucose 6-phosphate.

2.   Glucose phosphate isomerase catalyses this inter-conversion of isomers.

3.   The phosphofructokinase reaction, an important regulatory site, is modulated negatively by ATP and citrate and positively by AMP. Another ATP is consumed in the reaction.

3.   The aldolase reaction results in the splitting of a hexose bisphosphate into two triose phosphates.

4.   The isomers glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (DHAP) are interconverted by the enzyme triosephosphate isomerase. In an isolated system the equilibrium favors DHAP formation. However, in the cellular environment it is shifted completely toward the production of glyceraldehyde 3-phosphate, since this metabolite is being continuously removed

from the equilibrium by the subsequent reaction catalysed by glyceraldehyde 3-phosphate dehydrogenase.

5.   In this reaction, glyceraldehyde 3-phosphate is oxidised to a carboxylic acid, while inorganic phossphate is incorporated as a high-energy anhydride bond. The enzyme is glyceraldehyde 3-phosphate dehydrogenase, which uses NAD as its hydrogennaccepting cosubstrate. Under aerobic conditions, the NADH formed is reoxidised to NAD by O_2 via the electron transport chain in the mitochondria. The reason the O_2 is not necessary to sustain this reaction under anaerobic conditions is that the NAD consumed is restored by a subsequent reaction (see 11 below).

6.   This reaction, catalysed by phosphoglycerate kinase, exemplifies a substrate level phosphorylation of ADP. Do a little extensive reading, for a more detailed review of this mechanism by which ATP can be formed from ADP by the transfer of a phosphate from a high-energy donor molecule.

7.   Phosphoglyceromutase catalyses the transfer of the phosphate group from the carbon-3 to carbon-2 of the glyceric acid.

8.   Dehydration of 2-phosphoglycerate by the enzyme enolase introduces a double bond that imparts high energy to the phosphate bond.

9.   The product of reaction (9), phosphoenolpyruvate (PEP), donates its phosphate group to ADP in a reaction catalysed by pyruvate kinase. This is the second site of substrate level phosphorylation of ADP in the glycolytic pathway.

10.   The lactate dehydrogenase reaction transfers two hydrogen from NADH and H+ to pyruvate, reducing it to lactate. NAD is formed in the reaction and can replace the NAD consumed in reaction (6) under anaerobic conditions. It must be emphasised that this reaction is most active in situations of oxygen debt, as in prolonged muscular activity. Under normal, aerobic conditions, pyruvate enters the mitochondrion for complete oxidation. A third important option available to pyruvate is its conversion to the amino acid alanine through trans-amination with the amino group donor glutamate. This, together with the fact that pyruvate is also the product of the catabolism of various amino acids, makes it an important link between protein and carbohydrate metabolism.

11. These two reactions provide the means by which dietary fructose enters the glycolytic pathway. Fructose is an important factor in the average American diet, since nearly half of the carbohydrate consumed is sucrose, and high fructose corn sugar is becoming more popular as a food sweetener. Reaction 12 functions in extrahepatic tissues and involves the direct phosphorylation by hexokinase to form fructose 6-phosphate. This is a relatively unimportant reaction. It is slow and occurs only in the presence of high levels of the ketose. Reaction 13 is the major means by which fructose is converted to glycolysis metabolites.

The phosphorylation occurs at carbon-l and is catalysed by fructokinase, an enzyme found only in hepatocytes. The fructose I-phosphate is subsequently split by aldolase, designated aldolase B to distinguish it from the enzyme acting on fructose 1,6-bisphosphate, forming DHAP and glyceraldehyde. The latter can then be phosphorylated by glyceraldehyde kinase (or triokinase) at the expense of a second ATP to produce glyceraldehyde 3-phosphate. Fructose is therefore converted to glycolytic intermediates and as such can follow the pathway to pyruvate formation and Krebs cycle oxidation. Alternatively, they can be used in the liver to produce free glucose by a reversal of the first part of the pathway through the action of gluconeogenic enzymes. Glucose formation from fructose would be particularly important if fructose provides the major source of carbohydrate in the diet.
Since the phosphorylation of fructose is essentially the responsibility of the liver, the ingestion of large amounts of the ketose can cause a depletion of hepaatocyte ATP, leading to reduction in the rate of various biosynthetic processes such as protein synthesis.

12   Like glucose and fructose, galactose is first phosphorylated. The transfer of the phosphate from ATP is catalysed by galactokinase and the resulting phosphate ester is at carbon-I of the sugar. The major dietary source of galactose is lactose, from which the monosaccharide is hydrolytically released by lactase.

13.   Galactose 1-phosphate can be converted to glucose I-phosphate by the enzyme galactose 1-phosphate uridyl transferase. The reaction involves the transfer of a uridyl phosphate residue from UDP glucose to the galactose I-phosphate, yielding glucose 1-phosphate and UDP galactose. As glucose 1-phosphate, galactose can be incorporated into glyycogen through reactions discussed previously. It can enter the glycolytic pathway following isomerisation to glucose 6-phosphate and be hydrolysed to free glucose in liver cells.

14.   This indicates the entry of glucose 6-phosphate into another pathway called the hexose monophosphate shunt (pentose phosphate pathway), which will be considered next.

3.7   Hexosemonophosphate Shunt

The purpose of a shunt is to generate biochemically important intermediates not produced in other pathways. Two consecutive dehydrogenase reactions, glucose 6-phosphate dehydrogenase (G-6-PD), and 6-phosphogluconate dehydrogenase, initiate the sequence of reactions. Both reactions require NADP as cosubstrate. Consequently, a large amount of NADPH is formed, and this reduced co substrate is used for other important metabolic functions, such as the biosynthesis of fatty acids and the maintenance of reducing substances in red blood cells necessary to ensure the functional integrity of the cells. The shunt also provides pentose sugars necessary for the synthesis of DNA and RNA.

This is achieved by the decarboxylation of 6-phosphogluconate to form the pentose phosphate ribulose 5-phosphate, which in turn is converted to its aldose isomer, ribose 5-phosphate. In some cells the pathway ends at this point. In other cells, three-, four-, and seven-carbon phosphate sugars are subsequently formed. Through molecular re-arrangements catalysed by the fragmentring enzymes transketolase and transaldolase, fructose 6-phosphate is ultimately produced, serving as a "return" to the glycolytic sequence of reactions, thus completing the shunt.
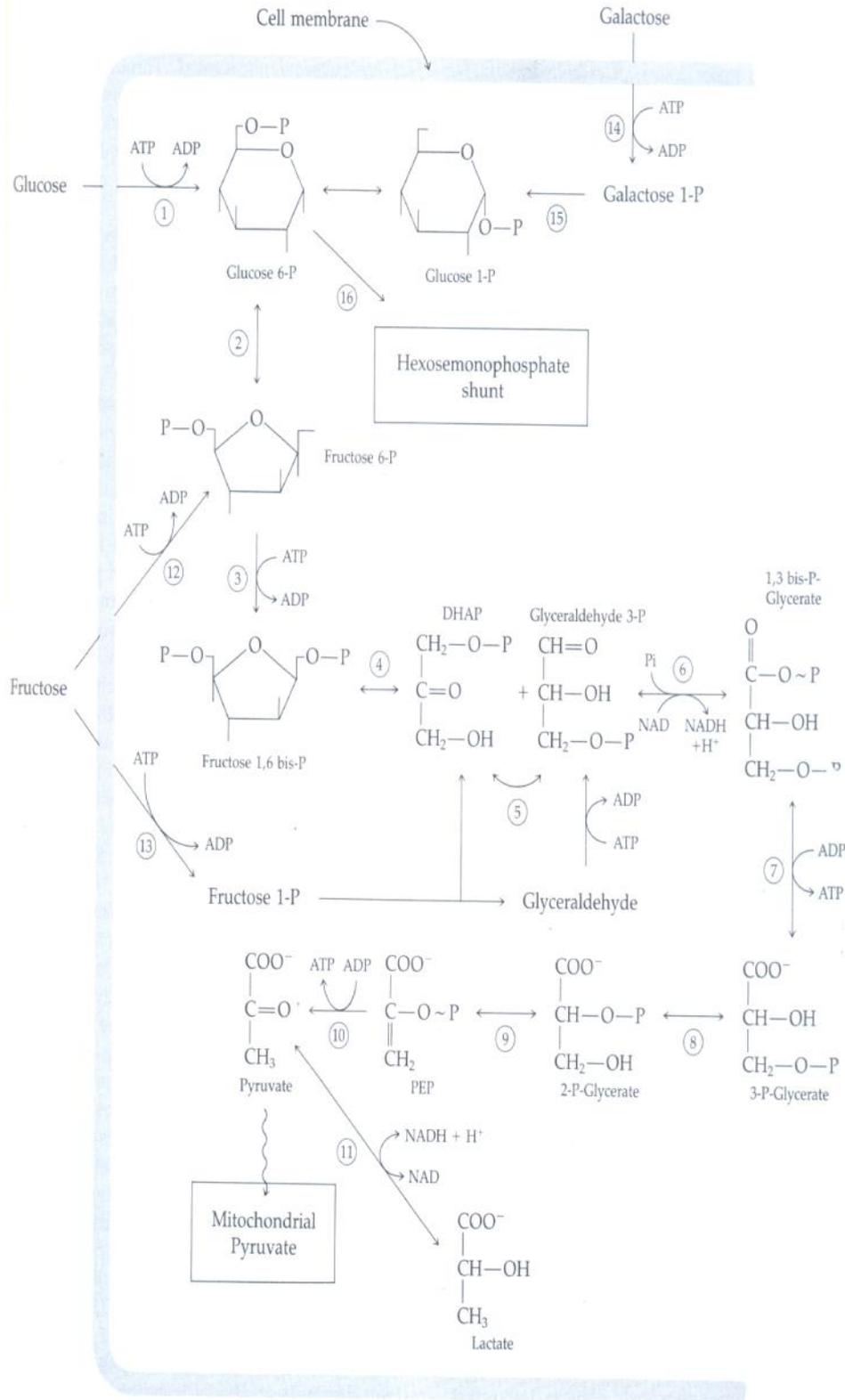
Fig.2.6:Glycolysis, Indicating The Mode of Entry of Glucose, Fructose, and Galactose into the Pathway, as Well as the Alternative Digression Of Glucose 6-Phosphate into the Hexosemonophosphate Shunt.

The shunt is active in liver, adipose tissue, adrenal cortex, thyroid gland, testis, and lactating mammary gland. Its activity is low in skeletal muscle because of the limited demand for NADPH (fatty acid synthesis) in this tissue and also due to muscle's reliance on glucose for energy.

Under anaerobic conditions, the progression of glucose to lactic acid is of low energy yield from the standpoint of ATP formed. This is predictable on the basis of structural change alone, noting that only one carbon-carbon bond was cleaved in converting a hexose into two trioses, and only one reaction was an oxidation reaction. The one NADH produced does not undergo re-oxidation via mitochondrial electron transport, since molecular oxygen is the ultimate oxidising agent in that system. Instead it is used in the lactate dehydrogenase reduction of pyruvate to lactate. In the glycolytic pathway, therefore, a net two ATPs are formed, one is consumed in each of the reactions one and three, but two are produced by substrate level phosphorylation at each of the reactions seven and 10 because two triose phosphate substrates were formed from one glucose molecule.

When the system is operating aerobically and the supply of oxygen is ample to effect total oxidation of incoming glucose, lactic acid is not formed. Instead, pyruvate enters the mitochondrion, as does a reducing equivalent of the NADH (see below) produced in reaction six. The latter becomes oxidised by electron transport and consequently generates three ATPs/mol NADH by oxidative phosphorylation. Therefore, six additional moles of ATP are formed, assuming two triose units for each glucose, bringing the total to eight. NADH cannot enter the mitochondrion directly. Rather, reducing equivalents formed from the NADH in the cytoplast are shuttled across the mitochondrial membrane and in turn reduce intramitochondrial NAD to NADH.

Shuttle substances that transport the hydrogens removed from cytosolic NADH into the mitochondrion are malate or glyceroI3-phosphate. The major shuttle compound, malate, is reoxidised by malate dehydrogenase within the mitochondrion as NAD becomes reduced to NADH, therefore generating three ATPs/mol, as discussed above. The glycerol 3phosphate shuttle, on the other hand, leads to only two ATPs/mol NADH because the intramitochondrial reoxidation of the glycerol 3-phosphate is cattalysed by glycerol phosphate dehydrogenase, which uses FAD instead of NAD as hydrogenacceptor. If the glycerol 3-phosphate shuttle is in effect, therefore, a total of only six ATPs will be formed under aerobic conditions through the glycolytic sequence-two by substrate level phosphorylation and four by oxidative phosphorylation. These shuttle systems function in the reoxidation of cytoplasmic NADH.

If the starting point of glycolysis is glycogen rather than free glucose, the hexokinase reaction is bypassed, and the total energy yield is therefore increased by one ATP for either aerobic or anaerobicglycolysis.

CH_2—O—P

Glucose 6-phosphate

G-6-PDH

NADP$^+$

NADPH + H$^+$

CH_2—O—P

=O 6-phosphoglucono-δ-lactone

Lactonase

COO$^-$

CH—OH

HO—CH

CH—OH

CH—OH

CH_2—O—P

6-phosphogluconate

NADP$^+$

6-PGDH

H$^+$ + NADPH

CO$_2$

CH_2—OH

C=O

CH—OH

CH—OH

CH_2—O—P

D-ribulose
5-phosphate

Phosphopentose
isomerase

CH=O

CH—OH

CH—OH

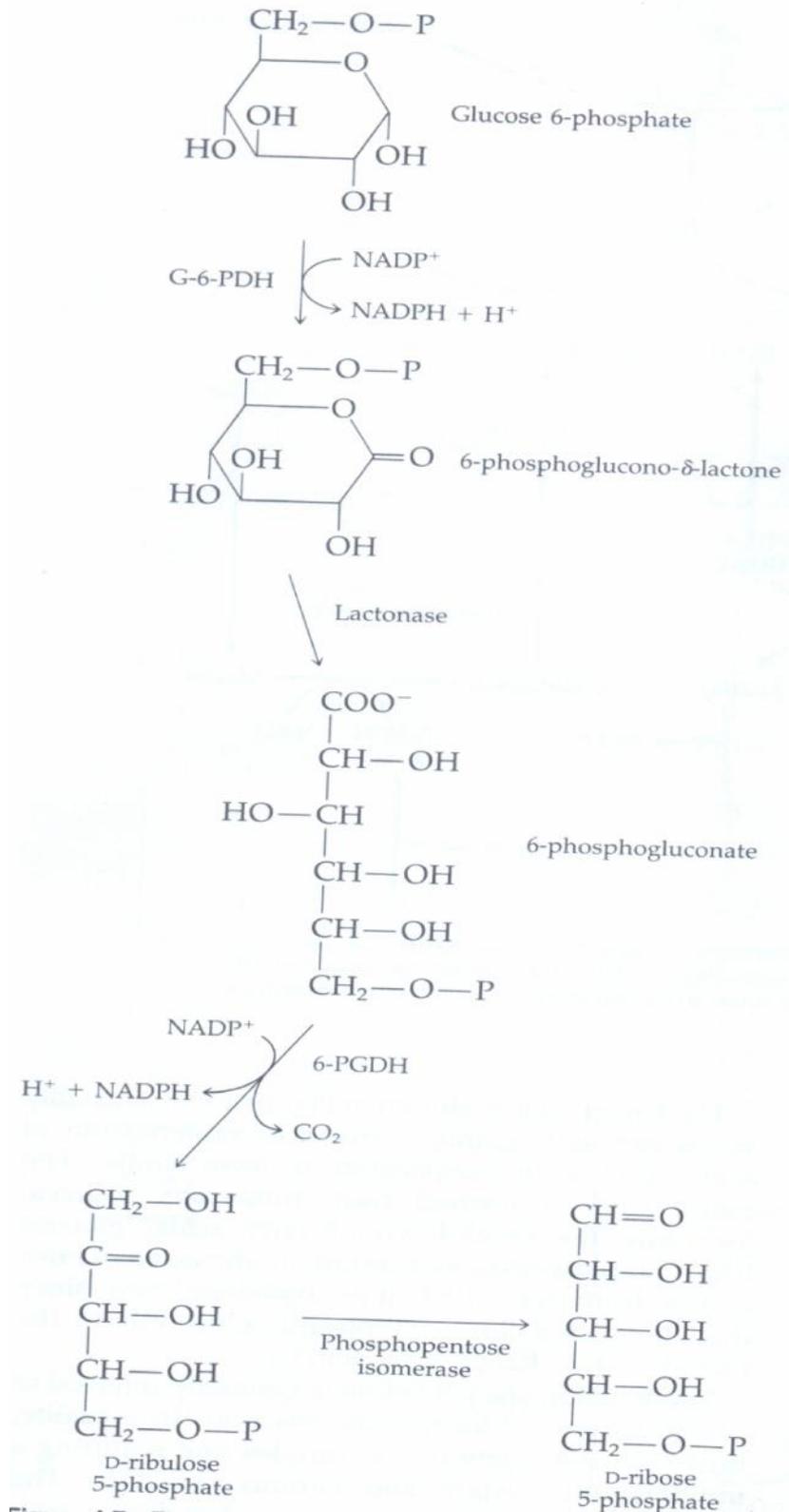CH—OH

CH_2—O—P

D-ribose
5-phosphate

Fig.2.7:*The Portion of The Hexosemonophosphate Shunt Showing the Generation of NADPH By The G 6-PDH (Glucose 6-Phosphate Dehydrogenase) And 6-PGDH (6-Phosphogluconate Dehydrogenase) Reactions. Adding to The Importance of The Latter Reaction is That it also forms Pentose Phosphates by The Decarboxylation of 6 – Phosphogluconate.*

3.8   Krebs Cycle

Alternatively designated the tricarboxylic acid cycle or the citric acid cycle, this sequence of reactions represents the forefront of energy metabolism in the body. It can be thought of as the common and final catabolic pathway because products of carbohydrate, fat, and amino acids feed into the cycle where they can be totally oxidised to CO_2 and H_2O, with the accompanying generation of large amounts of ATP. Not all entrant substances are totally oxidised. Some Krebs cycle intermediates are used to form glucose by the process of gluconeogenesis, which will be discussed in the next section, and some can be converted to certain amino acids by transamination. However, the importance of the cycle as the nucleus of energy production is evidenced by the estimation that over 90 per cent of energy released from food occurs here.

The high energy output of the Krebs cycle is attributed to mitochondrial electron transport, with oxidative phosphorylation providing the means for ATP formation. The oxidation reactions occurring in the cycle are actually dehydrogenations in which an enzyme catalyses the removal of two hydrogens to an acceptor co-substrate such as NAD or FAD. Since the enzymes of the cycle and the enzymes and electron carriers of electron transport are both compartmentalised within the mitochondria, the reduced cosubstrates, NADH and FADH2 are readily reoxidised by O2 via the electron transport chain.
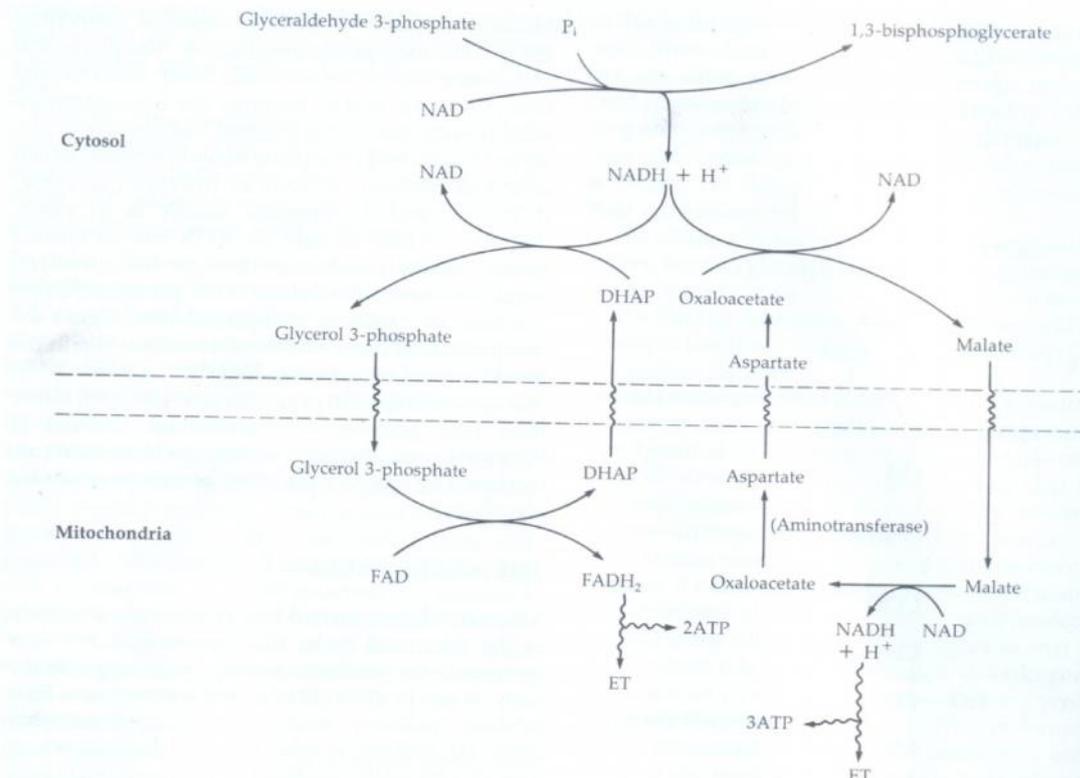
Fig.2.8:This is The Krebs (Citric Acid) Cycle. This Representation of the Cycle is Designed to Emphasise The Formation of Reduced Coenzymes and How Their Reoxidation by Electron Transport Contributes to the Synthesis of ATP.

In addition to its production of the reduced co-substrates NADH and FADH2, which furnish the energy through their oxidation via electron transport, the Krebs cycle produces most of the carbon dioxide through decarboxylation reactions. Viewing this in its proper perspective with regard to glucose metabolism, it must be recalled that two pyruvates are produced from one glucose during cytoplasmic glycolysis. These pyruvates are in turn transferred into the mitochondria, where decarboxylation leads to the formation of two acetyl CoA units and two mollecules of CO_2. The two carbons represented by the acetyl CoA are additionally lost as CO_2 through Krebs cycle decarboxylations. Most of the CO_2 produced is exhaled through the lungs, although some is used in certain synthetic reactions called carboxylations.

The Krebs cycle is shown in figure below. It is usually visualised as beginning with the condensation of acetyl CoA with oxaloacetate to form citrate. The acetyl CoA is formed from numerous sources, including the breakdown of fatty acids, glucose (through pyruvate), and certain amino acids. Its formation from pyruvate will be considered now, since this compound links cytoplasmic glycolysis to the mitochondrial Krebs cycle activity.

The reaction shown below is generally referred to as the pyruvate dehydrogenase reaction. In actuality, however, the reaction is a complex one requiring a multienzyme system and various cofactors. The enzymes and cofactors are contained within an isolable unit called the pyruvate dehydrogenase complex. The cofactors include coenzyme A (CoA), thiamine diphosphate (TDP), Mg^{+2}, NAD, FAD, and lipoic acid. Four vitamins are therefore necessary for the activity of the complex- pantothenic acid (a component of CoA), thiamine, niacin, and riboflavin. The role of these vitamins and others as precursors of coenzymes will be discussed in another unit. The enzymes include pyruvate decarboxylase, dihydroolipoyl dehydrogenase, and dihydrolipoyl transacetylase. The net effect of the complex results in decarboxylation and dehydrogenation of pyruvate with NAD serving as the terminal hydrogen acceptor. This reaction therefore yields energy, since the reoxidation by electron transport of the NADH produces three mol of ATP by oxidative phosphorylation. The reaction is regulated negatively by ATP and by NADH.

The condensation of acetyl CoA with oxaloacetate initiates the Krebs cycle reactions. The following are comments on reactions:

1. The formation of citrate from oxaloacetate and acetyl CoA is catalysed by citrate synthetase. The reaction is regulated negatively by ATP. The isomerisation of citrate to isocitrate involves cis aconitate as an intermediate. The isomerisation, catalysed by aconitase, involves dehydration followed by sterically reversed hydration, resulting in the repositioning of the -OH group onto an adjacent carbon. The first of four dehydrogenation reactions within the cycle, the isocitrate dehydrogenase reaction supplies energy through the respiratory chain reoxidation of the NADH. Note that the first loss of CO_2 in the cycle occurs at this site. It arises from the spontaneous decarboxylation of an intermediate compound, oxalosuccinate. The reaction is positively modulated by ADP and negatively modulated by ATP and NADH.
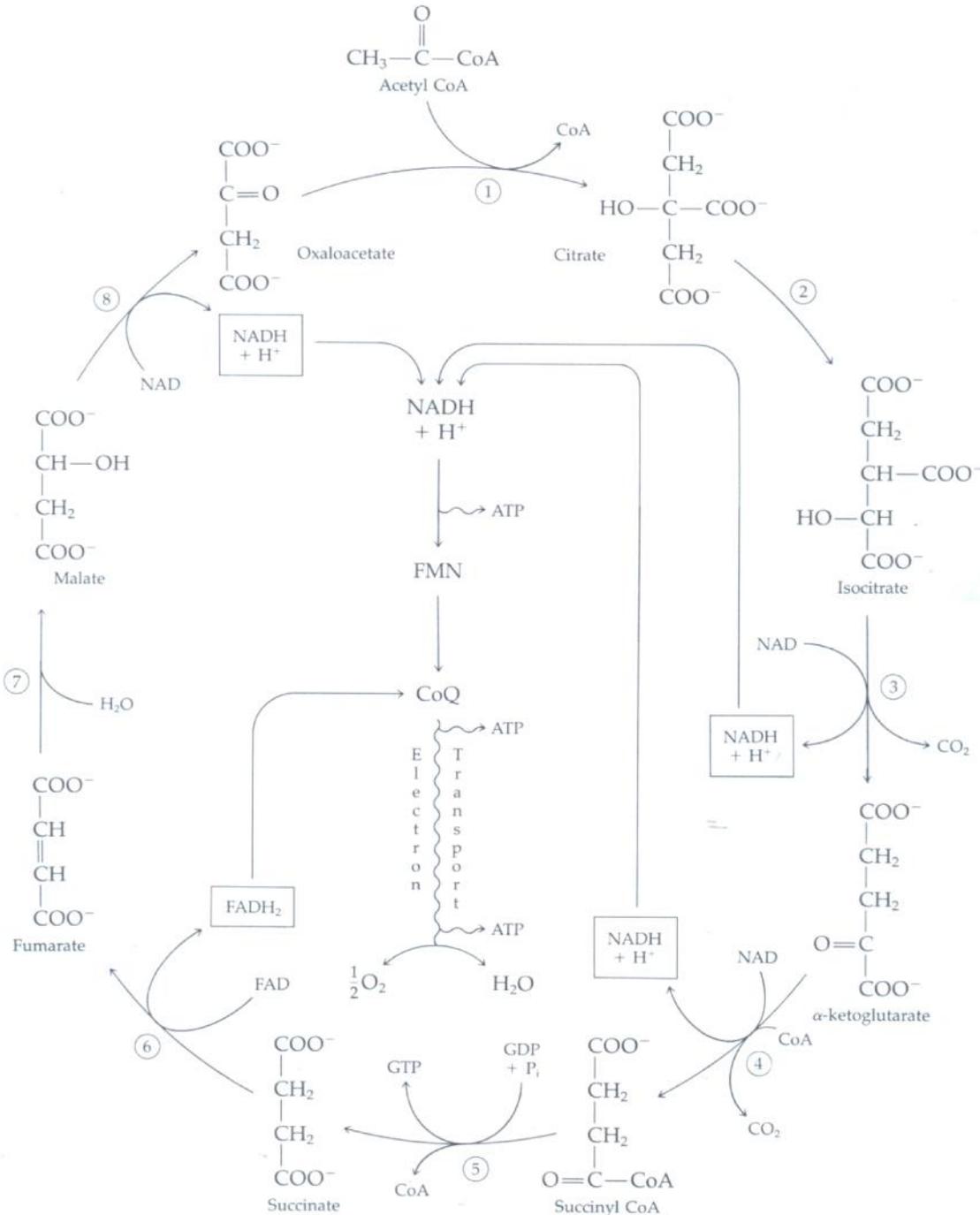
Fig.2.9:The Krebs (Citric Acid) Cycle. This Representation of the Cycle is Designed to Emphasise The Formation of Reduced Coenzymes and how their Reoxidation by Electron Transport Contributes to the Synthesis of ATP.

2.　　The decarboxylation/dehydrogenation of aglutarate is mechanistically identical to the pyruvate dehydrogenase complex reaction in its multi-enzyme/cofactor requirement. In the reaction, referred to as the -ketoglutarate dehydrogenase reaction, NAD serves as hydrogen acceptor, and a second carbon is lost as CO_2.

The pyruvate dehydrogenase, isocitrate dehydrogenase, and aglutarate dehydrogenase reactions account for the loss of the three-carbon equivalent of pyruvate as CO_2.

3.   Energy is conserved in the thioester bond of succcinyl CoA. The hydrolysis of that bond by succinyl thiokinase releases sufficient energy to drive the phosphorylation of guanosine diphosphate (GDP) by inorganic phosphate. The resulting GTP is a high energy phosphate anhydride compound like ATP; as such, GTP can serve as phosphate donor in certain phosphorylation reactions. One such reaction occurs in the gluconeogenesis pathway.

4.   The succinate dehydrogenase reaction uses FAD instead of NAD as hydrogen acceptor. The FADH2 is reoxidised by electron transport to O2, but only two ATPs are formed by oxidative phosphorylation instead of three.

5.   Fumarase incorporates the elements of H_2O across the double bond of fumarate to form malate.

6.   The conversion of malate to oxaloacetate completes the cycle. NAD acts as a hydrogen acceptor in this dehydrogenation reaction catalysed by malate dehydrogenase. It is the fourth site of reduced co substrate formation and therefore of energy release in the cycle.

In summary the complete oxidation of glucose to CO_2 and H_2O can be shown by the equation:
$$C_6H_{12}O_6 + 6O_2 \quad 6\,CO_2 + 6\,H_2O + energy.$$

This is achieved by the combined reaction sequences of the glycolytic and Krebs cycle pathways. The amount of released energy conserved as ATP under aerobic conditions is as follows:

The glycolytic sequence, glucose   2 pyruvates, produces two ATPs by substrate level phosphoryllation and either four or six by oxidative phosphoorylation, depending on the shuttle system for NADH-reducing equivalents (p. 86). Generally, six will be formed due to the overall greater activity of the malate shuttle system. The intra mitochondrial pyruvate dehydrogenase reaction yields two *mol* of NADH, one for each pyruvate oxidised and therefore six additional ATPs by oxidative phosphorylation.
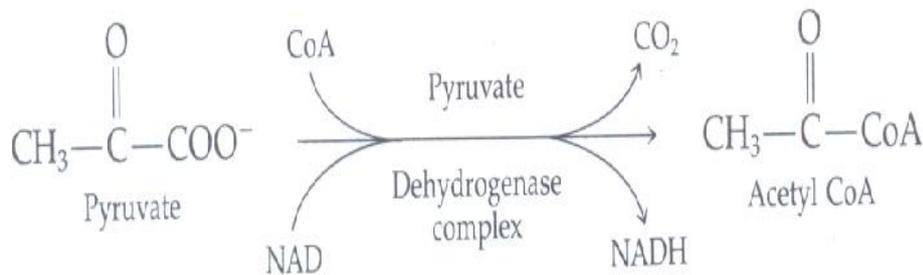
Fig.2.10

The oxidation of 1 mol of acetyl CoA in the Krebs cycle yields a total of 12 ATPs. The sites of formation, indicated by reaction number, follow.

3. 3 ATP
4. 3 ATP
5. 1 ATP (as GTP)
6. 2 ATP
8. 3 ATP

Total   12 ATP

Since 2 mol acetyl CoA derived from one glucose, however, the actual total is 24 ATPs. The total number of ATPs realised for the complete oxidation of 1 mol of glucose is therefore 38, equivalent to 262.8 kcal. It will be recalled that this figure represents only about 40% of the total energy released by mitochondrial electron transport. The remaining 60 per cent, or approximately 394 kcal, is released as heat to maintain body temperature.

It has already been mentioned that acetyl CoA is produced by fatty acid oxidation and amino acid catabolism as well as from the glycolytically derived pyruvate. This clearly leads to an imbalance between the amount of acetyl CoA and oxaloacetate, which condense one to one stoichiometrically in the citrate synthetase reaction. It is therefore important that oxaloacetate and/or Krebs cycle intermediates, which can form oxaloacetate, be replenished in the cycle. Such a mechanism does indeed exist. Oxaloacetate, fumarate, succinyl CoA, and arate can all be formed from certain amino acids, but the single most important mechanism for ensuring an ample supply of oxaloacetate is the reaction by which it is formed directly from pyruvate. This reaction, shown below, is catalysed by pyruvate carboxxylase. The "uphill" incorporation of CO2 is accomplished at the expense of ATP, and the reaction requires the participation of biotin. The diversion of pyruvate into oxaloacetate is called an anaplerotic (filling up) process because of

its role in restoring oxaloacetate to the cycle. It is of interest that pyruvate carboxylase is regulated positively by acetyl CoA, thereby accelerating oxaloacetate formation in answer to increasing levels of acetyl CoA.
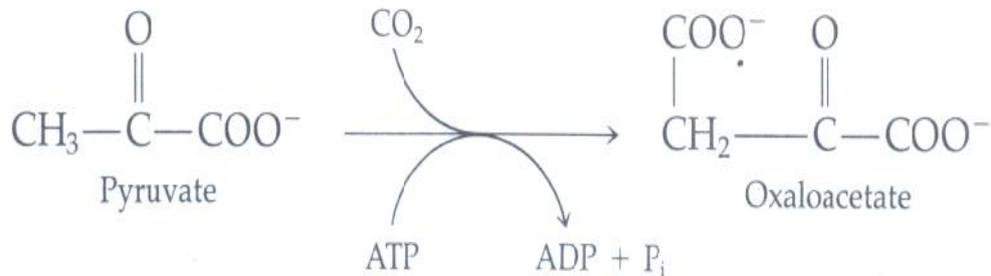

Fig.2.11

3.9   Gluconeogenesis

D-glucose is an essential nutrient for the proper function of most cells, particularly those of the brain and other tissues of the central nervous system (CNS). When dietary intake of carbohydrate is reduced and blood glucose concentration declines, a hormonal triggering of accelerated glucose synnthesis from noncarbohydate sources occurs. Lactate, pyruvate, glycerol (a catabolic product of triglycerrides), and certain amino acids represent the important noncarbohydrate sources. The process of producing glucose from such compounds is termed gluconeogenesis. The liver is the major site of this activity, although under certain circumstances, such as starvation, the kidney becomes increasingly important in gluconeogenesis. Muscle and adipose tissue lack the enzymes necessary for the process. This means, of course, that muscle lactate cannot serve as a precursor of glucose within that tissue. How, then, is the high level of muscle lactate that can be encountered in situations of oxygen debt dealt with? The lactate is transported to the liver via the general circulation, where it is able to be converted to glucose. The glucose can then be returned to the muscle cells to re-establish homeostatic concentrations there. This circulatory transport of muscle-derived lactate to the liver and the return of glucose to the muscle is referred to as the Cori cycle.

Gluconeogenesis is essentially a reversal of the glycolytic pathway. Most of the cytoplasmic enzymes involved in the conversion of glucose to pyruvate catalyse their reactions reversibly and therefore provide the means for also converting pyruvate to glucose. There are three reactions in the glycolytic sequence that are not reversible-the hexokinase, phosphofructokinase, and pyruvate kinase reactions. They are unidirectional by virtue of the high, negative-free energy change of the reactions. Therefore, the process of gluconeogenesis requires that these

reactions be bypassed by other enzyme systems. It is the presence or absence of these circumventing enzymes that determines if a certain organ or tissue is capable or incapable of conducting gluconeogenesis. As shown below in 4K, the hexokinase and phosphofructokinase reactions are bypassed by specific phosphatases that hydrolyse phosphate esters.

The bypass of the pyruvate kinase reaction involves the formation of oxaloacetate as an intermediate. Mitochondrial pyruvate can be converted to oxaloacetate by pyruvate carboxylase, the reaction that has been discussed as an anaplerotic process. Oxaloacetate can, in turn, be decarboxylated and phosphorylated to phosphoenolpyruvate by PEP carboxykinase, thereby completing the circumvention of the pyruvate kinase reaction. PEP carboxykinase is a cytoplasmic enzyme, and it is consequently necessary for oxaloacetate to leave the mitochondrion, the membrane of which, however, is impermeable to the -keto acid. It therefore must be converted to either malate (by malate dehydrogenase) or aspartate (by transamination with glutamate;), either of which freely traverse the mitochondrial membrane.

In the cytoplasm, the malate or aspartate can be converted to oxaloacetate by malate dehydrogenase or aspartate aminotransferase (glutamate oxaloacetate transaminase) respectively. This mechanism allows the carbon skeletons of various amino acids to enter the gluconeogenic pathway and lead to a net synthesis of glucose. Such amino acids are accordingly called glucogenic. They can be metabolically converted to pyruvate and to Krebs cycle intermediates. As such, they can ultimately leave the mitochondrion in the form of malate or aspartate, as discussed. Reactions showing the entry of non-carbohydrate substances into the gluconeogenic system along with the bypass of the pyruvate kinase reaction will be discussed later.

During the past decade, evidence has emerged from in vitro studies that glucose, as the sole substrate at physiologic concentrations, has limited use by the liver and is, in fact, a poor precursor of liver glycogen. However, use is greatly enhanced if gluconeogenic substances such as fructose, glycerol, or lactate are available along with the glucose. The facile incorporation of glucose into glycogen in vivo, but its limited conversion in vitro, has been referred to as the glucose paradox. It is one of many examples of the importance of interactions among nutrients.

4.0   CONCLUSION

The cellular use of carbohydrates depends on their absorption from the gastrointestinal (GI) tract into the blood stream, a process normally restricted to monosaccharides. Therefore, polysaccharides and

disaccharides must be hydrolysed to their constituent monosaccharide units. The maintenance of normal blood-glucose concentration is the net effect of metabolic processes that remove glucose from the blood for either glycogen synthesis or for energy production and of processes that return glucose to the blood.

5.0 SUMMARY

In this unit, you have learnt the following:

Dietary starches and disaccharides are ultimately hydrolysed completely by specific glycosidases to constituent monosaccharide residues that are capable of being absorbed from the intestine.

The control and regulation of the metabolic pathways, is accomplished by: hormonal induction or activation of specific enzymes; negative or positive modulation of allosteric enzymes by effector compounds; and shifts in reaction equilibria by changes in reactant or product concentrations.

6.0 TUTOR-MARKED ASSIGNMENT

1. What is the metabolic fate of the monosaccharides in the body?
2. Using necessary chemical structures, describe the citric acid cycle.
3. Write short notes on the following:
    a. Glycogenesis
    b. Glycogenolosis
    c. Glycolysis
    d. Hexose monophosphate shunt
    e. Gluconeogenesis.

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UNIT 3   STRUCTURE,   PROPERTIES   AND CLASSIFICATION OF PROTEINS

 CONTENTS

1.0   Introduction
2.0   Objectives
3.0   Main Content
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                3.1.1   Primary Structure
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                3.1.4   Quaternary Structure
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        3.3   Classification of Proteins
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1.0   INTRODUCTION

This unit will highlight the structure of proteins (primary, secondary, tertiary and quaternary), properties of proteins and classification of proteins (simple, conjugated and derived). Proteins are a group of compounds containing carbon, hydrogen, oxygen, nitrogen (about 16%) and sulphur. In some proteins, phosphorus or iron is present and occasionally may contain iodine, copper and zinc. Proteins are found in all living cells in plants and animals, where they are intimately connected with all phases of activity that constitute the life of the cell. Each species has its own specific proteins, and a single organism has many different proteins in its cells and tissues. It follows therefore that a large number of proteins occur in nature. All proteins are made up basically of amino acids and there are 20 standard amino acids in nature. The proteins differ from each other in the number of sequence of these standard amino acids.

2.0   OBJECTIVES

By the end of this unit, you will be able to:

- highlight structures of protein, properties of protein
- explain the major classification of protein.

3.0   MAIN CONTENT

3.1   Structure of Proteins

Protein, as a substance has different frameworks. For your proper understanding, we shall present that structure of proteins under primary, secondary, tertiary and quaternary proteins.

3.1.1   Primary Structure

This structure comes to existence as a consequence of the linkage between the - Carboxyl of one amino acid and the - amino group of another acid.
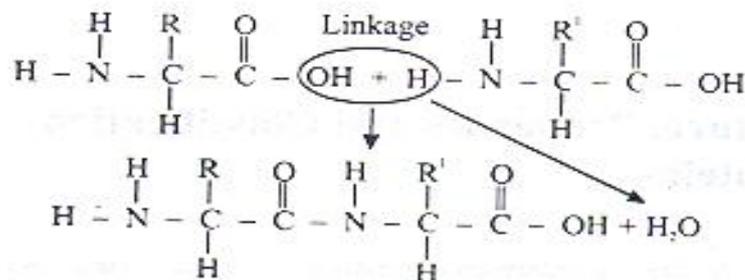
Fig.3.1

This type of linkage is known as peptide linkage and it is a linkage formed between two amino acids followed by the elimination of water as revealed in above structures. In the illustrated structure, a dipeptide was formed from two amino acids. Numerous amino acids can be married together using this addition procedure. When this happened with the removal of one molecule of H20 at every linkage, the polypeptides are produced.
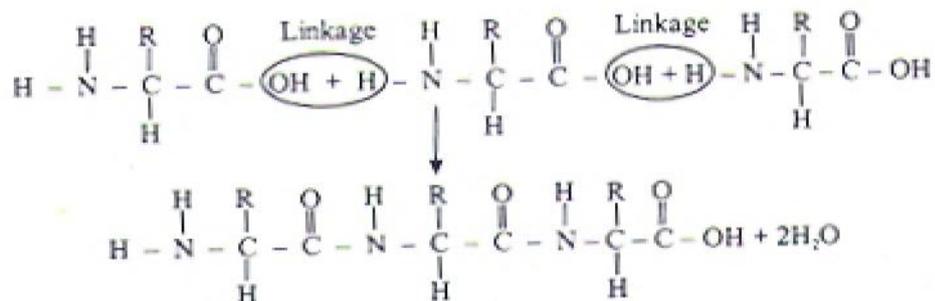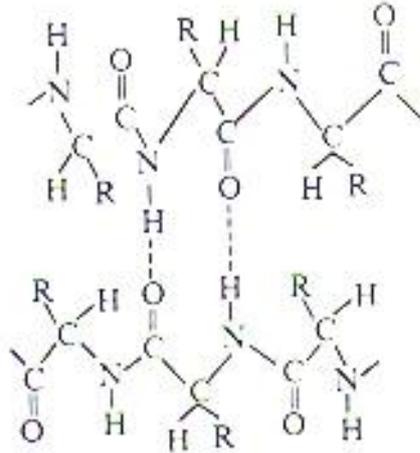
Fig.3.2:Polypeptide

3.1.2 Secondary Structure

This structure shows the conformation of the chain of amino acids emanating due to production of hydrogen bonds between the amino (NH) and carboxyl groups of adjacent amino acids as illustrated below:

Fig.3.3

3.1.3 Tertiary Structure

This structure is formed as a result of further interaction of secondary structure through the R groups of the amino acid residues. Such emanated interaction predisposes polypeptide chain to folding and bending.

3.1.4 Quaternary Structure

Proteins have this structure if they contain more than one polypeptide chain. The agents that equilibrate these combinations are hydrogen bonds and salt bonds produced between residues on the surfaces of polypeptide chains.

3.2   Properties of Proteins

3.2.1 Colloidal

Proteins are peculiar in their water solubility. Keratin and albumins are insoluble and soluble proteins respectively. Soluble proteins can be precipitated from solution and such precipitation can in turn be redissolved.

3.2.2 Amphoteric

All proteins possess a certain amount of free amino and carboxyl groups, either as terminal units or in the side-chain of amino acid residues.

3.2.3 Denaturation

All proteins can be altered or dephased from their natural occurrence. It is a chemical, physical and biological alteration of a unique structure of proteins. Coagulation of a protein solution upon heating, shrinking of meat when heated or fired and roasting of nuts are few examples of denaturation of protein.

3.3   Classification of Proteins

Proteins can conveniently be grouped on the basis of both physical (shape) and chemical properties. The physical properties commonly employed for grouping are those of solubility and heat coagulation. Standing on these two characteristics; proteins are classified into three as simple, conjugated and derived proteins.

3.3.1 Simple Proteins

Simple proteins are the proteins that yield only amino acids or their derivatives when hydrolysed. Examples are many which include albumins, globulins, glutelins, albminoids, histones and prolamins. It is important we briefly examine these simple proteins.

Albumins

They are soluble in water and coagulable by heat. They are found both in plants and animals, e.g. myosin of muscle, serum albumin of blood and lactalbumin of wheat.

Globulins

These are not soluble in pure water but could be dissolved in solution of alkaline and acid. They are heat coagulated. They generally contain glycine. Globulin constitutes an important and widely distributed group of animal and plant proteins, for example, ovoglobulin of egg yolk, myosin of muscle, phaseolin of beans, legumins of peas and arachin of peanuts.

Glutelins

Axe all plant proteins and soluble in very dilute acids and alkalis, but they are insoluble in natural solvents.

Prolamins

Prolamins are soluble in alcohol but insoluble in water or neutral solvents. These proteins generally yield proline and amide nitrogen upon hydrolysis but are deficient in lysine. Prolamins are plant proteins found principally in seeds, e.g. zein of corn, hadein of barley, gleaden of wheat.

Albuminoids

It is the least soluble of all the proteins. They are generally insoluble in water, dilute acids, alkalis and alcohol. They are entirely animal protein and are the chief constituents of skeletal structures such as hair, horn, hoof, and nails. They are also constituents of supporting and connecting of fibrous tissues and of the cartilage and bone.

Histones

The proteins are soluble in water and insoluble in dilute ammonia. They are readily soluble in dilute acids and alkali. They are not readily coagulated by heat. Histones are basic proteins. They yield a large proportion of basic amino acids upon hydrolysis. They often precipitate other proteins from solution.

Protamins

Protamins are strongly basics and yield mainly basic amino acids on hydrolysis particularly arginine. They are soluble in water, dilute ammonia acid and alkalis. They are not coagulated by heat. They precipitate other proteins from their solution.

3.3.2 Conjugated Proteins

Conjugated proteins are composed of simple proteins combined with non-proteins substance. The non-protein group is referred to as prosthetic group or addition group. The types of conjugated proteins include the following:

i.   **Nucleo Proteins**: They composed of simple basic protein (protamin or histone) in salt with nucleo acid or nucleic. They are

proteins of cell and apparently the chief constituents of chromatin. These are the most abundant in tissues of both plants and animals, having a large proportion of nucleic materials such as yeast, thymus and other glandular organs.

ii. **Mucoproteins or Mucoids**: The mucoproteins are composed of simple proteins combined with mucopolysaccharide such as hyaluronic acid, chomdrotin sulphates. They generally contain large amount of N-acetylated henosamine and in addition + or - of such substances are uronic acid, sialic acid and monosaccarid. Water soluble mucoproteins have been obtained from human urine, serum and egg white. Each water soluble mucoprotein are not easily denatured by heat or readily precipitated by agents such as picric acid and trichloro acetic acid.

iii. **Chromoproteins**: These proteins are composed of simple proteins united with coloured prosthetic group. Many proteins of important biological function belong to this group. Examples of chromoproteins are:

iv. **Haemoglobin**: Respiratory proteins in which the prosthetic group is iron containing prophysm called EME.

v. **Cytochromes**: These are cellular of oxidation, reduction protein in which the prosthetic group is also HEME.

vi. **Flavoproteins**: They are cellular oxidation-reduction proteins in which the prosthetic groups are riboflavin.

vii. **Visual purple of the retina**: It is a chromoprotein in which the prosthetic group is carotenoid pigment.

viii. **Phosphoproteins**: Phosphoric acid is the prosthetic group of phosphoprotein. Phosphosenine has been isolated from casein (milk) and vitellin (egg).

ix. **Lipoproteins**: Lipoproteins are formed by combination of proteins with lipid such as lecithin, cephalin, and fatty acid, etc. phospholipid proteins are widely distributed in plants and animals, milk and in chloroplast of plant.

x. **Metalloproteins**: This is a large group of enzyme proteins which contain metallic element such as Fe, Co, Mn, Zn, Cu, Mg, etc. which are parts of their essential structures.

3.3.3 Derived Proteins

This class of proteins, as the name implies, includes those substances derived from simple and conjugated proteins. It is the least well defined of the protein groups. Derived proteins are subdivided into primary derived proteins and secondary derived proteins.

Primary derived proteins

These protein derivatives are formed by processes which cause only slight changes in the protein molecule and its properties. There is little or no hydrolytic cleavage of peptide bonds. The primary derived proteins are synonymous with denatured proteins.

i.   **Proteans:** The proteans are insoluble products formed by the incipient action of water, very dilute acids, and enzymes. They are particularly formed from certain globulins, but differ from globulins in being insoluble in dilute salt solutions. In general, they have the physical characteristics of the naturally occurring glutelins. Examples include myosan from myosin, edestan from edestin, and fibrin from fibrinogen.

ii.   **Metaproteins:** The metaproteins are formed by further action of acids and alkali upon proteins. They are generally soluble in very dilute acids and alkali but insoluble in neutral solvents. Examples include acid and alkali metaproteins such as acid and alkali albuminates.

iii.   **Coagulated proteins:** The coagulated proteins are insoluble products formed by the action of heat or alcohol upon natural proteins. Similar substances may also be formed by action of ultraviolet light, x-rays, very high pressure, and mechanical shaking upon protein solutions at the isoelectric pH. Examples include cooked egg albumin, cooked meat and other proteins, and alcohol-precipitated proteins.

Secondary derived proteins

These substances are formed in the progressive hydrolytic cleavage of the peptide unions of protein molecules. They represent a great complexity of molecules of different sizes and amino acid composition. They are roughly grouped into proteoses, peptones, and peptides, according to relative average molecular complexity. Each group is composed of many different substances.

i.   **Proteoses or albumoses:** Proteoses are hydrolytic products of proteins which are soluble in water, are not coagulated by heat,

and are precipitated from their solutions by saturation with ammonium sulfate.

ii.   **Peptones:** Peptones are hydrolytic products of simpler structures than the proteoses. They are soluble in water, are not coagulated by heat, and are not precipitated by saturation with ammonium sulfate. They are precipitated by phosphotungstic acid.

iii.   **Peptides:** Peptides are composed of only a relatively few amino acids united through peptide bonds. They are named according to the number of amino acid groups present as di-, tri-, tetra peptides or polypeptides. They are water-soluble, are not coagulated by heat, are not salted out of solution, and are often precipitated by phosphotungstic acid. Various definitely characterised peptides have been isolated from protein hydrolytic products, and many have been synthesised.

The complete hydrolytic decomposition of a natural protein molecule into amino acids generally progresses through successive stages as follows:

protein   protean   metaproteins
proteose   peptone   peptides   amino acids

The synthesis of proteins by plants and animals consists of a progressive process in which amino acid groups are successively joined by peptide linkages until the molecular size and structure is that of a specific plant or animal protein. On the other hand, in protein catabolism, the proteins of tissues are continually being broken down to amino acids through the various hydrolytic stages. Accordingly, substances belonging to the classes of proteoses, peptones, and peptides are constituents of tissues, though often in very small amounts.

4.0   CONCLUSION

Proteins are compounds containing carbon hydrogen, oxygen, nitrogen sulphur and phosphorus. They are found in all living cells (plants + animals) where they are connected will all phases of activity that constitute life of the cell.

5.0   SUMMARY

In this unit, you have learnt that**:**

Proteins possess four basic structures namely:

- Primary
- Secondary
- Tertiary
- Quaternary Protein

Protein can be classified into the following classes:

- Simple proteins
- Conjugated proteins
- Derived proteins

Protein serves different functions that include the following:

- Provision of major organic structure of major organic structure of the protoplasmic mechanic
- Involves in chemical process of food digestion
- Storage of amino acids and their usage as building blocks.
- Transportation of some specific molecules via the blood.

6.0   TUTOR-MARKED ASSIGNMENT

1.   a.   What are proteins?
      b.   List 20 hydrolytic products of protein.
2.   a.   Enumerate the structures of proteins.
      b.   Identify the inherent characteristics of proteins.
3   Based on the hydrolysis products of proteins, describe the three major classes of   proteins.

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UNIT 4   METABOLISM OF PROTEINS AND NUCLEIC ACIDS

CONTENTS

1.0   INTRODUCTION

The digestion of dietary protein by gastric and intestinal proteases liberates amino acids which are readily absorbed into the portal circulation. This can be demonstrated by measurement of total amino nitrogen of the plasma. Between meals, the normal plasma amino nitrogen level is 4-6 mg/100ml, but during the absorption of a protein meal the amino nitrogen increases by 2-4 mg/100ml.

The amino acids are rapidly taken up by the tissues particularly the liver, intestine and kidney. By the sixth or seventh hour, the plasma level returns to the base line value. The lymph is not an important route of transport for amino acids. Free amino acids are not stored in the tissues to any great extent but are metabolised by incorporation into protein or by transamination or deamination and further oxidation. Reserves of protein accumulate in the liver and possibly in the muscle. These labile

reserves, which can be called upon when the protein intake is inadequate, are incorporated into the architecture of the tissues. The liver is the site of synthesis of several blood proteins, e.g. plasma, albumin, globulins, fibrinogen and oprothrombin. The liver metabolises any amino acids excess of hepatic needs for protein synthesis. The liver does this by converting the nitrogen atoms to intermediates encountered in carbohydrate and lipid metabolism.

2.0   OBJECTIVES

By the end of this unit, you will be able to:

- explain the digestion of dietary protein by gastric and intestinal proteases
- explain the amino acids turn-over of body proteins.

3.0   MAIN CONTENT

3.1   Enzymatic Digestion of Dietary Proteins

The degradation of dietary proteins in vertebrates occurs in the gastrointestinal tract. Entry of dietary protein in the stomach stimulates the gastric mucosa to secrete the hormone gastrin, which in turn stimulates the secretion of hydrochloric acid by the parietal cells and pepsinogen by the chief cells of the gastric glands. The acidic gastric juice (pH 1.0-2.5) functions both as an antiseptic, killing most bacteria and foreign cells and as a denaturing agent, unfolding globular proteins and rendering their internal peptide bonds more accessible to enzymatic hydrolysis. Pepsinogen (an inactive precursor/zymogen) is converted to active pepsin. In the stomach, pepsin hydrolyses ingested proteins at the peptide bonds on the amino-terminal side of the aromatic amino acid residues phenylalanine, tryptophan and tyrosine, cleaving long peptide chains into a mixture of smaller peptides.

As the acidic content pass into the small intestine, the low pH triggers secretion of the hormone secretin into the blood. Secretin stimulates the pancreas to secrete bicarbonate into the small intestine to neutralise the gastric HCl, abruptly increasing the pH to about 7. The digestion of proteins continues in the small intestine as the arrival of amino acids in the upper part of the small intestine (duodenum) causes the release of the hormone cholecystokinin into the blood. This stimulates secretion of several enzymes: trypsinogen, chymotrypsinogen, procarboxypeptidases A and B, the zymogens of trypsin, chymotrypsin and carboxypeptidases A and B which are synthesised and secreted by the exocrine cells of the pancreas. Trypsinogen is converted to its active form, trypsin by enteropeptidase, a proteolytic enzyme secreted by intestinal cells. Free

trypsin then catalyses the conversion of additional trypsinogen to trypsin as well as activates chymotrypsinogen, procarboxypeptidase A and B and proelastase.

Trypsin and chymotrypsin further hydrolyse the peptides produced by pepsin in the stomach. Trypsin cleaves the peptides at the carbonyl side of lysine and arginine while chymotrypsin cleaves at the carbonyl side of the amino acids phenylalanine, tryptophan and tyrosine. Degradation of the short peptides in the small intestine is completed by other intestinal peptidases; carboxypeptidases A and B which remove successive carboxyl-terminal residues from peptides and an aminopeptidase that hydrolyses successive amino-terminal residues from short peptides. The resulting mixture of free amino acids is transported into the epithelial cells lining the small intestine, through which the amino acids enter the blood capillaries in the villi and travel to the liver.

3.2   Amino Acid Catabolism

Excess amino acids are not stored or excreted as amino acids, but are rather degraded. Amino acids undergo degradation in three different metabolic circumstances:
1.   During normal body metabolism, some amino acids that are released from protein breakdown and are not needed for new protein synthesis undergo oxidative degradation

2.   When a diet is rich in protein and the ingested amino acids are in excess of the body's needs for protein synthesis, the surplus is catabolised

3.   During starvation or in uncontrolled diabetes mellitus, when carbohydrates are unavailable or not properly utilised, cellular proteins are broken down and used as fuel (energy).

The degradative pathways for most amino acids begin by the removal of the -amino nitrogen by the process of deamination. Deamination occurs by two major routes: transamination and oxidative deamination.

Transamination

This step involves the loss of the amino group of an amino acid to form a -ketoacid (the carbon-skeletons of amino acids). The amino group is taken on by -ketoglutarate (an intermediate of the citric acid cycle) to form glutamate. This reaction is highly reversible because the reacting functional groups of the product are identical to those of the reactants. This reaction is mediated by glutamate aminotransferase. The co-

enzyme, pyridoxal phosphate (PLP) is present at the catalytic site of aminotransferases and serves as a carrier of amino groups.
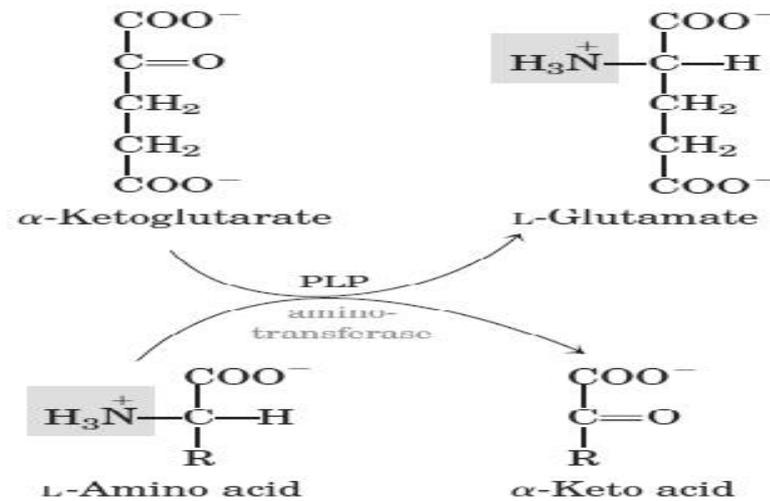

Fig.4.1

Oxidative Deamination of L-glutamate

The release of -amino nitrogen as ammonia from L-glutamate is catalysed by L-glutamate dehydrogenase (GDH) which can use either nicotinamide adenine dinucleotide (NAD^+) or nicotinamide adenine dinucleotide phosphate ($NADP^+$) as a co-substrate. This reaction is also freely reversible but favours glutamate production. It involves hydride transfer from glutamate to NAD^+, leading to -iminoglutarate imine followed by hydrolysis to -ketoglutarate.
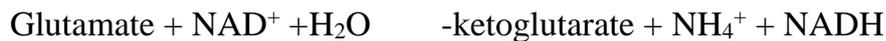
Glutamate + NAD^+ +H_2O -ketoglutarate + NH_4^+ + NADH
The - ketoglutarate formed from this step can be used in the citric acid cycle and for glucose synthesis.   The combined action of an aminotransferase and glutamate dehydrogenase is referred to as transdeamination.
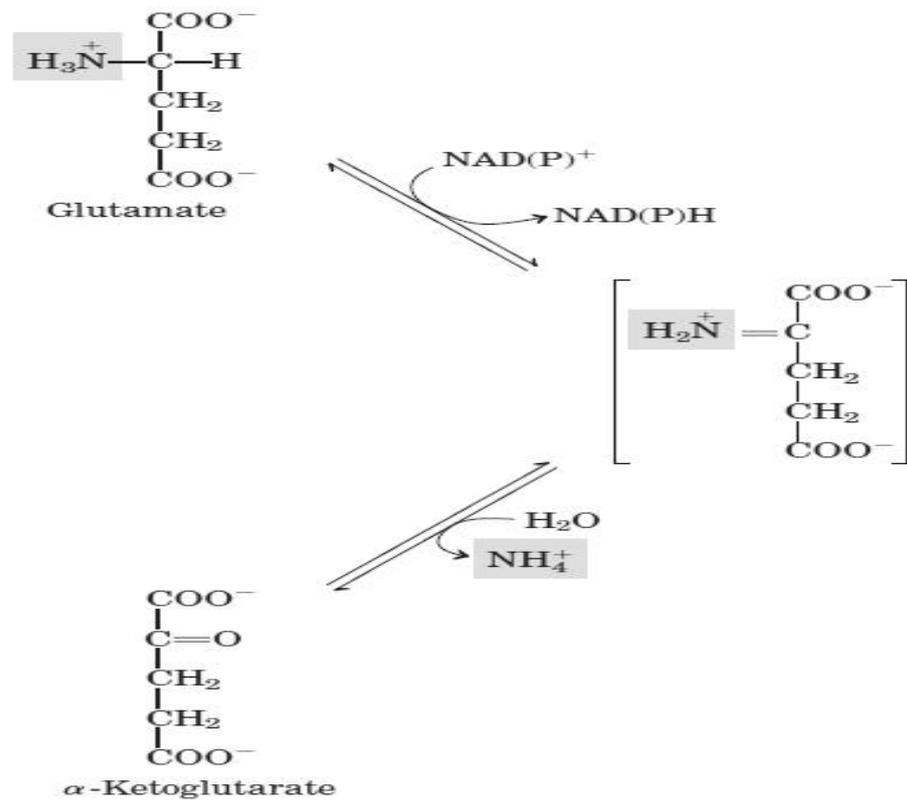
Fig.4.2

3.3   Ammonia Transport in the Blood

Ammonia is quite toxic to animal tissues and the level present in the blood is regulated. In most animals, much of the free ammonia is converted to a nontoxic compound before export from the extrahepatic tissues into the blood and transfer to the liver or kidney. For this transport function, the free ammonia is combined with glutamate to yield glutamine by the action of glutamine synthetase. This reaction requires ATP and occurs in two steps.

First, glutamate and ATP react to form ADP and a -glutamyl phosphate intermediate, which then reacts with ammonia to produce glutamine and inorganic phosphate. Glutamine is a nontoxic transport form of ammonia and is normally present in blood in much higher concentrations than amino acids. It serves as a source of amino groups in a variety of biosynthetic reactions.
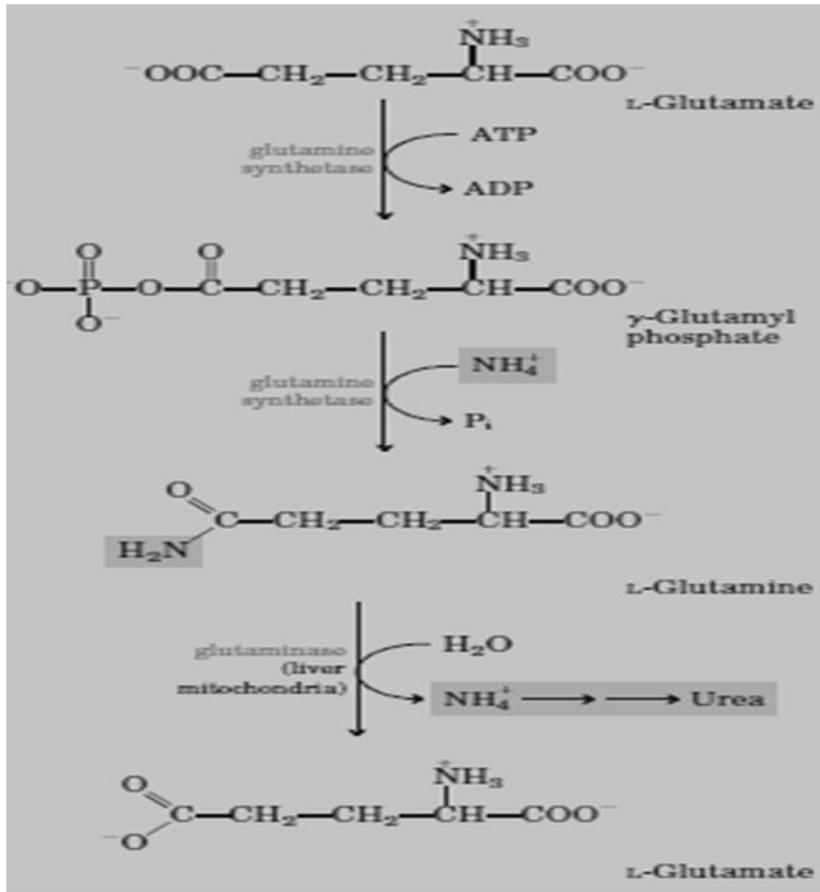
Fig.4.3

In most terrestrial animals, glutamine in excess of that required for biosynthesis is transported in the blood to the intestine, liver and kidneys for processing. In these tissues, the amide nitrogen in glutamine is released as ammonium ion in the mitochondria where the enzyme glutaminase converts glutamine to glutamate and NH_4^+. The NH_4^+ from intestine and kidney is transported in the blood to the liver. In the liver, the ammonia from all sources is disposed of by urea synthesis.

3.4   The Urea Cycle

The urea cycle is a mechanism for removing unwanted nitrogen. Urea formation in the liver starts with the multistep conversion of ornithine to arginine. This is followed by the breakdown of arginine into ornithine and urea. The complete urea cycle as it occurs in the mammalian liver requires five enzymes: argininosuccinate synthase, arginase and arginosuccinate lyase (which function in the cytosol), and ornithine transcarbamoylase, and carbamoyl phosphate synthase (which function in the mitochondria). Additional specific transport proteins are required for the mitochondrial uptake of L-ornithine, NH_3 and HCO_3^- and for the release of citrulline.

METABOLISM OF CARBOHYDRATES, LIPIDS, PROTEINS AND NUCLEIC ACIDS

The free ammonia formed at the end of oxidative deamination is converted into carbamoyl phosphate in a three-step reaction requiring two ATP molecules

$NH_4^+ + HCO_3^- + 2ATP$ carbamoyl phosphate $+ HPO_4^{2-} + 2ADP + 2H^+$
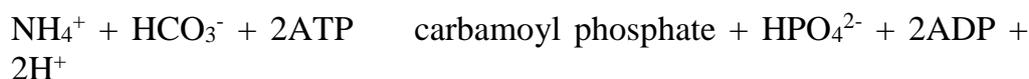
First, the bicarbonate is activated and subjected to nucleophilic attack by ammonia to form carbamoyl phosphate. This reaction is ATP dependent and catalyzed by carbamoyl phosphate synthase I. The carbamoyl phosphate now enters the urea cycle. The urea cycle has four enzymatic steps. First, carbamoyl phosphate donates its carbamoyl group to ornithine to form citrulline, with the release of Pi, ①. This reaction is catalysed by ornithine transcarbomoylase, and the citrulline passes from the mitochondrion to the cytosol.

Within the cytosol, citrulline reacts with L-aspartate in another ATP-dependent reaction to form argininosuccinate, AMP, and PPi, ②. The PPi is subsequently hydrolysed to inorganic phosphate, thus this step could be said to require 2 ATP molecules. This reaction is catalysed by argininosuccinate synthetase. The argininosuccinate is then cleaved by argininosuccinase to form free arginine and fumarate, ③. Fumarate enters into the mitochondria to join the pool of citric acid cycle intermediates, this is the only reversible step in the urea cycle. In the last reaction of the urea cycle, the enzyme arginase cleaves L-arginine to ornithine and urea, ④. While the ornithine is transported to the mitochondria to be reutilised in further rounds of urea cycle, urea diffuses into the bloodstream and is ultimately eliminated through the kidneys in the urine. The stoichiometry for the urea cycle is:
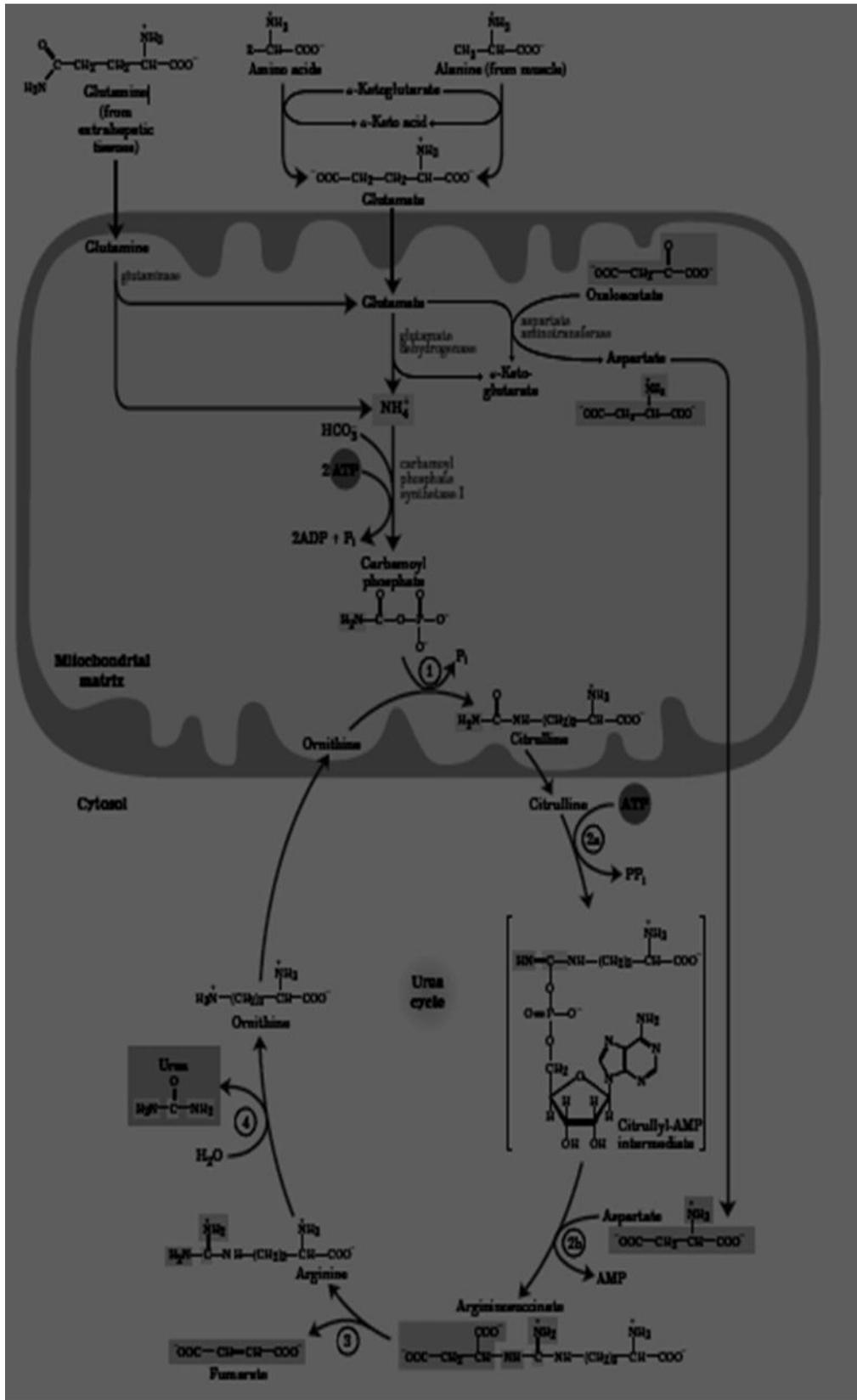
$CO_2 + NH_4^+ + 3ATP + Aspartate + 2H_2O$

Urea $+ 2ADP + 2P_i + AMP + PP_i +$ Fumarate $+ 6H^+$
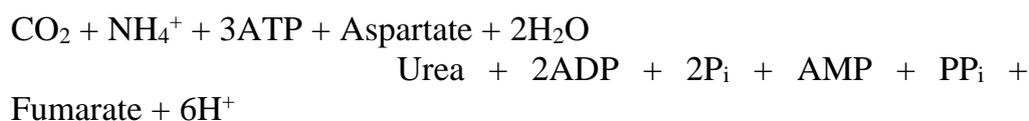
Fig.4.4

3.5   Excretory Forms of Nitrogen

Animals are not the only organisms that normally have a dietary excess of nitrogen but ammonia (which is the end product of nitrogen metabolism) is toxic to animals so must be gotten rid of. Excess nitrogen from amino acid breakdown is excreted on one of three forms depending on the availability of water.

Most aquatic vertebrates such as bony fishes and the larvae of amphibians release ammonia into the surrounding water where it gets diluted to non-toxic concentrations. This group of animals is called ammonotelic animals.

$$NH_4^+$$

Ammonia (as
ammonium ion)
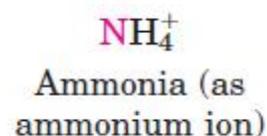
Terrestrial and aerial species convert ammonia to less toxic waste products that require little water for excretion. Most terrestrial vertebrates as well as sharks convert ammonia to urea in the liver synthesised by enzymes of the urea cycle and are called ureotelic animals.

$$H_2N—C—NH_2$$

Urea
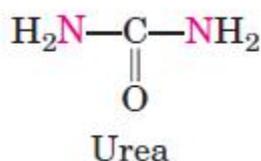
Birds and reptiles convert and ammonia and excrete uric acid as semisolid guano, hence they are called uricotelic animals.
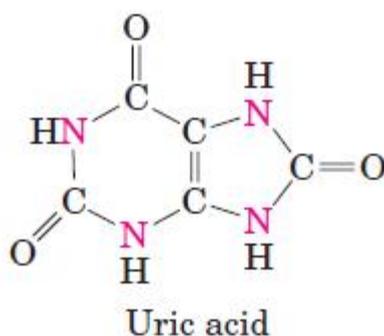
Uric acid

Fig.4.5

3.6   Intracellular Protein Turnover

The continuous synthesis and degradation of proteins in cells is collectively termed intracellular protein turnover, which determines

protein balance in tissues. Protein turnover requires large amounts of ATP (e.g., 20-25% of whole body energy expenditure in adults). However, this costly metabolic cycle fulfils key obligatory functions, including protein homeostasis, cell turnover, removal of aged and damaged proteins, synthesis of heat-shock and immunological proteins, gluconeogenesis, wound healing, tissue repair, adaptation to nutritional and pathological alterations, and immune responses.

When nitrogen losses equal intake, the subject is in nitrogen balance. Positive nitrogen balance which is characterised by an excess of nitrogen intake over loss, occurs during growth, repair of tissue losses (as in convalescence) and during pregnancy. Negative nitrogen balance, where losses exceed intake, is found in starvation, malnutrition, febrile diseases and after burns or trauma. During this period of negative nitrogen balance, the body may draw on its store of labile protein. When these are exhausted, circulating plasma protein will be depleted and hypoproteinemia results.

3.7   Metabolic Breakdown of Individual Amino Acids

Following the removal of amino groups from amino acids, their carbon skeletons are degraded to intermediates. The 20 individual catabolic pathways for protein amino acids converge to form only six major pathways, culminating in six intermediates all of which enter the citric cycle. These intermediates are oxaloacetate, -ketoglutarate, pyruvate, fumarate, succinyl CoA, acetyl CoA and acetoacetate. From here the carbon skeletons are diverted to gluconeogenesis or ketogenesis or are completely oxidised to CO_2 and H_2O.

Amino acids can be classified as glucogenic or ketogenic, or both based on which seven intermediates are produced during their catabolism. Glucogenic amino acids are amino acids that can be converted into glucose through gluconeogenesis. Their catabolism yield oxaloacetate, -ketoglutarate, pyruvate, fumarate, succinyl CoA. These are alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, histidine, methionine, threonine and valine.

Ketogenic amino acids are amino acids that can be converted to ketone bodies through ketogenesis. Their catabolism yield either acetoacetate or acetyl CoA. These are leucine and lysine. Some amino acids are both glucogenic and ketogenic and these are tyrosine, isoleucine, phenylalanine and tryptophan.
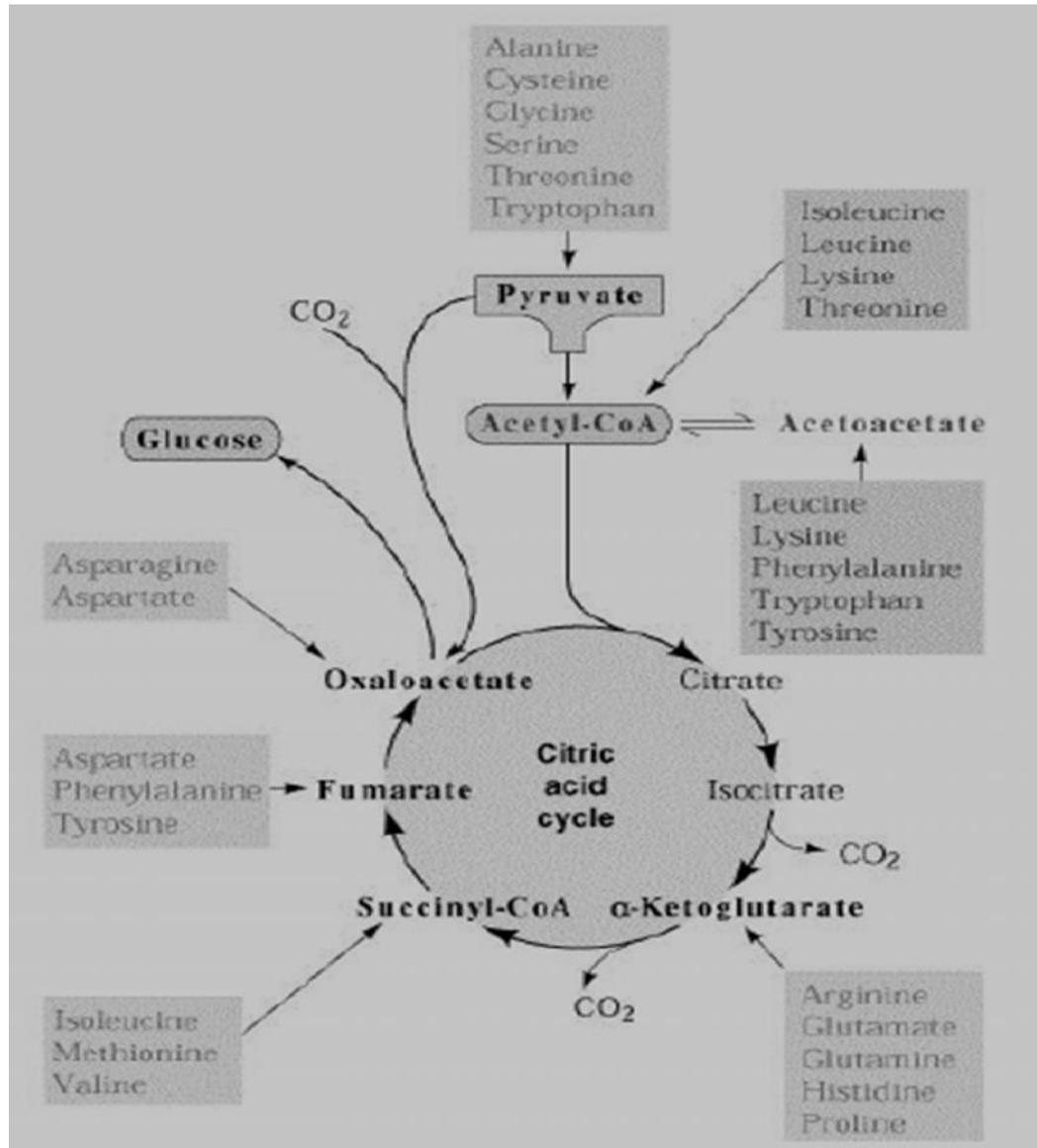
Fig.4.6:Metabolic Breakdown of Individual Amino Acids

3.8   Biosynthesis of Nutritionally Non-Essential Amino Acids

All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway. Nitrogen enters these pathways by way of glutamate and glutamine. Organisms vary greatly in their ability to synthesise the 20 common amino acids. Whereas bacteria and plants can synthesise all 20, mammals can synthesise only the non-essential amino acids, the essential amino acids must be obtained from food.

Nutritionally non-essential amino acids have very short biosynthetic pathways. The non-essential amino acids are formed by three general mechanisms.

3.8.1 Transamination

- Alanine can be synthesised by transamination of the corresponding -keto acid, pyruvate.

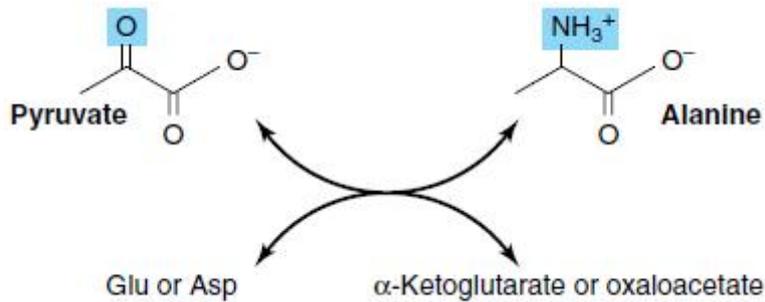 This reaction is catalysed by alanine aminotransferase.

Fig.4.7

- glutamate can be synthesised by transamination of the corresponding -keto acid, - ketoglutarate. This reaction is catalyzed by glutamate dehydrogenase
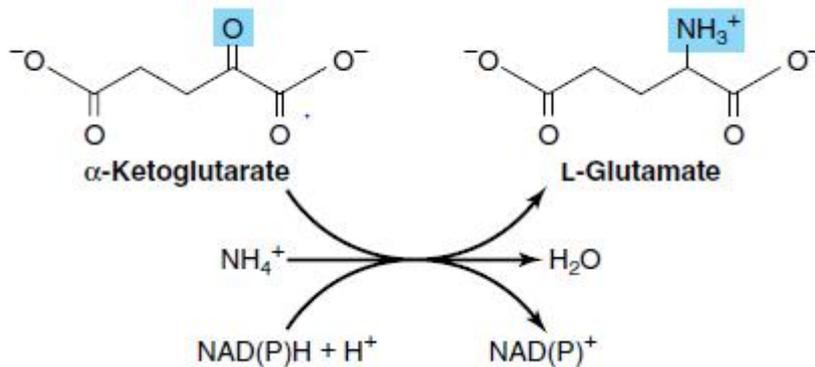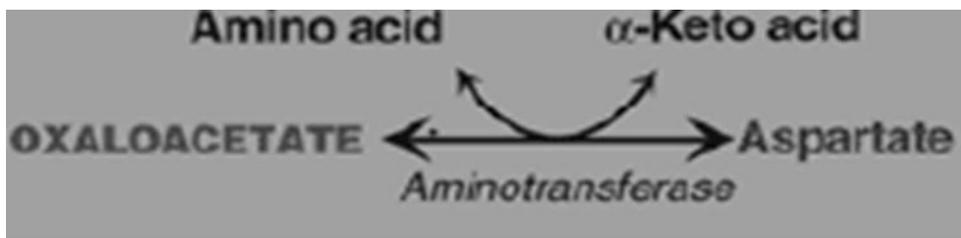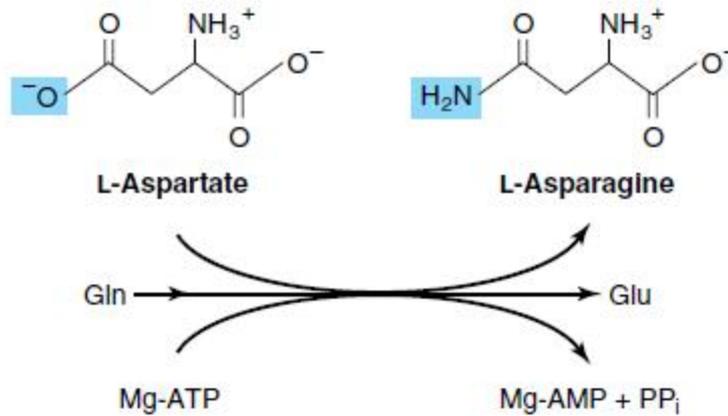

Fig.4.8

- Aspartate can be synthesised by transamination of the corresponding -keto acid, oxaloacetate. The conversion of aspartate to asparagine is catalyzed by asparagine synthetase.

Fig.4.9

- Serine can be synthesised by transamination and dephosphorylation of 3-phosphoglycerate, an intermediate of glycolysis. Oxidation of the -hydroxyl group of the glycolytic intermidiate 3-phosphoglycerate converts it to an oxo acid, whose subsequent transamination and dephosphorylation leads to serine.
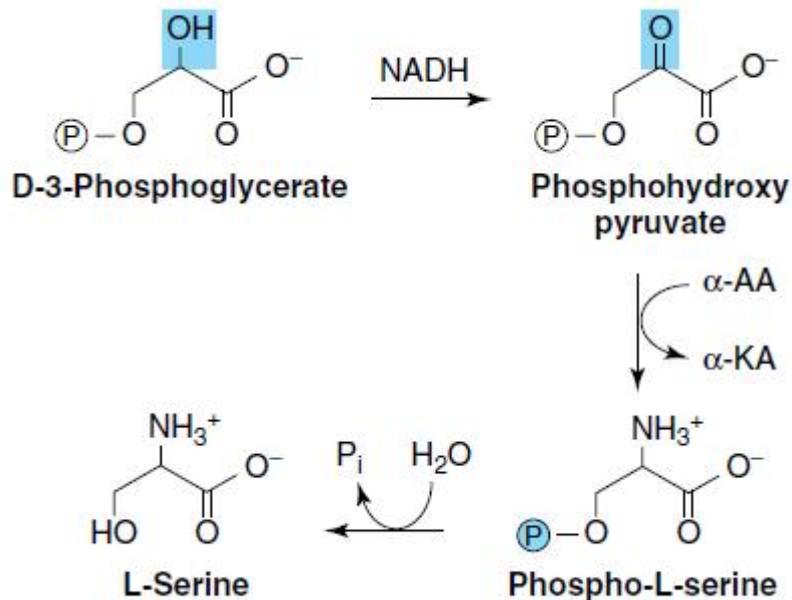
Fig.4.10

3.8.2 Assimilation of Free Ammonia

- **Glutamate:** Formation of glutamate from ammonia and -ketoglutarate is catalysed by glutamate dehydrogenase. This reaction is reversible and plays a role in both synthesis and breakdown of glutamate. Both NADPH and NADH can serve as the source of reducing equivalents used in this reaction.

- Glutamine: Glutamine synthetase catalyses the ATP-dependent formation of glutamine, using glutamate and ammonia as substrates.
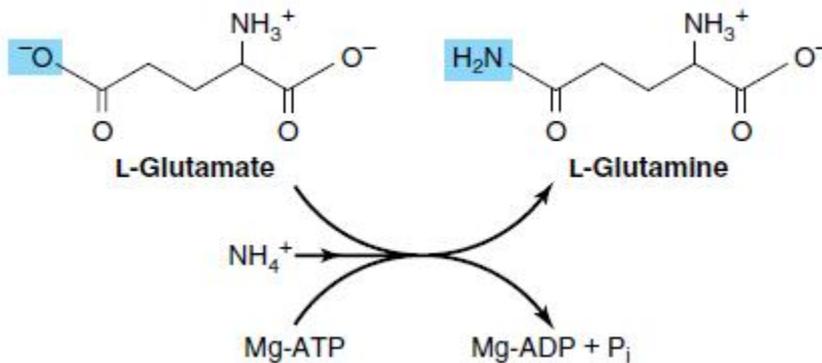
Fig.4.11

3.8.3 Modification of the Carbon Skeletons of Existing Amino Acids

- Cysteine: Cysteine contains atoms donated by both methionine and serine.
- Glycine: Serine is also converted to glycine by the removal of its hydroxymethyl group.
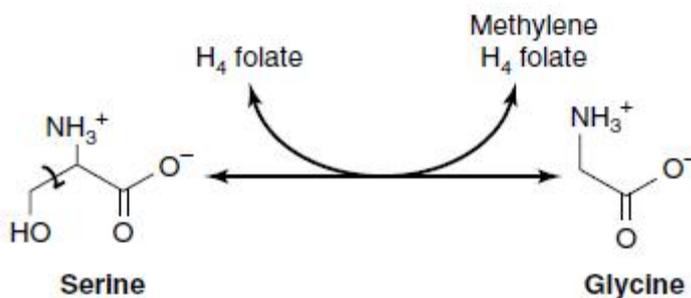
Fig.4.12

- Tyrosine: Phenylalanine is hydroxylated to form tyrosine.
- Proline: Glutamate is reduced and cyclised to form proline.
- Asparagine: Asparagine is synthesised by the transfer to the amide group of glutamine to the ß-carboxyl group of aspartate. The reaction is catalysed by asparagines synthetase.

3.9 Inborn Errors of Amino Acid Metabolism

3.9.1 Phenylketonuria: (PKU)

Phenylketonuria is the result of a deficiency in the enzyme phenylalanine hydroxylase. High levels of phenylalanine lead to competitive inhibition of the enzymes responsible for melanine production from tyrosine. Because little tyrosine converts to melanine, afflicted infants have blonde hair and fair skin and blue eyes (similar to albinism). Elevated phenylalanine, phenylpyruvate, phenylacetate, and phenyllactate are present in tissues, plasma and urine. The manifestations of the disease are mental retardation, failure to walk or talk seizures, hyperactivity and tremor.

3.9.2 Homocystinuria

Homocystinuria is due to a defect in the metabolism of homocysteine, deficiency of cystathionine synthase, high levels of homocysteine and methionine in plasma and urine. Patients exhibit ectopia (displacement of the lens of the eye), skeletal abnormalities, premature arterial disease, osteoporosis and mental retardation. Homocystinuria canbe treated by supplementation with high doses of vitamin B6, B12 and floate or restriction of methionine and inclusion of cysteine in the diet.

3.9.3 Albinism

Albinism is a condition in which defect in tyrosine metabolism results in deficiency in the production of melanin. Hypopigmentation caused due to the deficiency in the formation of melanine results in partial or full absence of pigment from the skin, hair, and eyes.

3.9.4 Alkaptonuria

This is a rare disease involving deficiency in homogentistic acid oxidase, enzyme in tyrosine degradation pathway, results in accumulation of homogentistic acidurea, large joint arthritis and dense, black pigments deposited on the intravertebral disks of the vertebrae.

4.0 CONCLUSION

The degradative pathways for most amino acids begin by the removal of the -amino nitrogen by the process of deamination. Deamination occurs by two major routes: transamination and oxidative deamination. Transamination plays an essential role in urea formation in connection with the synthesis of glutamate and aspartate as a means of transferring ammonia for the production

of urea. It should be noted that deamination and transamination are catabolic reactions while decarboxylation is anabolic (metabolic synthesis).

All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway. Nitrogen enters these pathways by way of glutamate and glutamine. Organisms vary greatly in their ability to synthesise the 20 common amino acids. Whereas bacteria and plants can synthesise all 20, mammals can synthesise only the non-essential amino acids, the essential amino acids must be obtained from food.

Inborn errors of amino acid metabolism are (i) **Phenylketonuria: (PKU)**, the result of a deficiency in the enzyme phenylalanine hydroxylase and is manifested by mental retardation, failure to walk or talk, seizures, hyperactivity and tremor**;** (ii) **Homocystinuria,** due to a defect in the metabolism of homocysteine, deficiency of cystathionine synthase, high levels of homocysteine and methionine in plasma and urine**;** (iii). **Albinism,** a condition in which defect in tyrosine metabolism results in deficiency in the production of melanin. Hypopigmentation caused due to the deficiency in the formation of melanine results in partial or full absence of pigment from the skin, hair, and eyes**;** and (iv). **Alkaptonuria, is** a rare disease involving deficiency in homogentistic acid oxidase, enzyme in tyrosine degradation pathway. This disorder results in accumulation of homogentistic acidurea, large joint arthritis and dense black pigments deposited on the intravertebral disks of the vertebrae.

5.0   SUMMARY

In this unit, you have learnt that the following**:**

The digestion of dietary protein by gastric and intestinal proteases liberates amino acids which are readily absorbed into the portal circulation. The amino acids are rapidly taken up by the tissues particularly the liver, intestine and kidney. At least three hormones are known to affect protein synthesis, pituitary growth hormone, insulin and testosterone.

All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway. They are required as the building blocks for the synthesis of the proteins of the blood and tissues. In addition, many of the amino acids are utilised in the formation of polypeptides and proteins of hormones and enzymes. Certain of the

amino acids found in proteins may be supplied preformed in the diet. These are termed the "nutritionally essentials amino acids". Other amino acids are synthesised in the tissues and these are known as the non-essential amino acids.

6.0   TUTOR-MARKED ASSIGNMENT

1.   Define metabolism.
2.   How are the absorbed nutrients distributed to the body tissues?
3.   What functions are performed by the absorbed amino acids?
4.   Describe the oxidation of amino acids in the body.
5.   What are the functions of the liver?
6.   Give an illustration of the action of a hormone.
7.   Describe the disposal of the various waste products of the body.

7.0   REFERENCES/FURTHER READING

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UNIT 5   LIPIDS METABOLISM

CONTENTS

1.0   INTRODUCTION

Lipids are organic compounds found in plant and animal tissue, and are oily/greasy substances. They are soluble in organic solvents such as benzene, ether or chloroform, but only sparingly soluble in water. In routine feed analysis, all kinds of lipids are determined together as the ether extract. Thus, the lipids include fats, oils, steroids, waxes, and other related compounds. Lipids are important dietary constituents not only because of their high energy value but also because of fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods.

2.0   OBJECTIVES

By the end of this unit, you will be able to:

- explain that the energy needed by the body is provided by the oxidation of CHOs and lipids
- discuss that the primary objective of lipid digestion is to arrange the lipids in a form that is water miscible
- explain that ketone body production only occurs during conditions of high circulating free fatty acids.

3.0   MAIN CONTENT

3.1   Definition of Terms

Lipids are biological molecules that are soluble in organic solvents. Lipids therefore include fats, oils, waxes, and related compounds. Occasionally, the term **lipoid** may be used in place of lipid. These two terms are synonymous and therefore can be used interchangeably.

Fat- a term referring either to the lipid in the foods or body fat, both of which are composed mostly of triglycerides

Triglyceride- the chief form of fat composed of C, H and O arranged as a molecule of glycerol with three fatty acids attached. The IUPAC (International Union of Pure and Applied Chemistry) name of triglyceride is triacylglycerol, which is the main storage form of fatty acids.

Glycerol- an organic alcohol composed of a 3carbon chain which can serve as the backbone for triglyceride.

Fatty acid- is a long chain organic acid having 4-24 carbon atoms with a single carboxyl group (COOH).

3.2   Importance of Lipids

Lipids have the following biological functions:
Certain lipids, fats, serve as efficient reserves for storage energy. Such lipids are found in the adipose tissues.

Fats serve as carriers for the fat soluble vitamins. The fat-soluble vitamins are, Vitamins A, D, E and K (A, D, E, K).

Lipids constitute the major structural element of membranes. When lipid is in combination with a protein the resulting substance is termed **lipoprotein,** i.e. lipid-protein.

Fat serves as insulating material in the subcutaneous tissues around certain organs.

Cholesterol, an example of a lipid, is a major substance from which Vitamin D and sex hormones are synthesised.

3.3   Types of Lipids

1)   Simple Lipids: esters of fatty acids with various alcohols; e.g. (a) Fats: esters of fatty acids with glycerol and (b) Waxes: esters of fatty acids with higher molecular weight monohydric alcohol

2)   Complex Lipids: esters of fatty acids containing groups in ddition to an alcohol and a fatty acid e.g. (a) phospholipids-lipids containing, in addition to fatty acid and an alcohol, a phosphoric acid residue. They frequently have nitrogen-containing bases and other substituents e.g., in glycerophoslipids the alcohol is glycerol and in sphingophospholipids the alcohol is spingosine. (b) Glycolipids: lipids containing a fatty acid and carbohydrate (c) Other complex lipids e.g. lipoprotein

3)   Derived lipids: these are substances derived from the hydrolysis of simple lipids and compound lipids. The derived lipids include fatty acids, glycerol, steroids, alcohols in addition to glycerol and sterols, fatty aldehydes and ketone bodies.

3.4   Functions of Lipids

i.   Lipids as an energy reserve: nearly all of the energy needed by the human body is provided by the oxidation of CHOs and lipids. Whereas CHOs provide a readily available source of   energy, lipids function primarily as energy reserve. The amount of lipids stored as an energy reserve far exceeds the energy stored as glycogen since the human body is simply not capable of storing as much glycogen compared to lipids. Lipids yields 9kcal of energy per gram while CHOs and proteins yield only 4kcal of energy per gram.

ii.   Protection of thermo-sensitive tissues against excessive heat loss to the environment.

iii.   Lipid serves as a thermal insulator in the subcutaneous tissues and around certain organs.

iv   Triacylglycerol also serves as the principal function of
        padding skeletons and vital organs, thus, protecting against
        shock. The heart, kidneys, epidydimis and mammary
        glands are enfolded by a layer of fat tissues.

v.   Phospholipids and cholesterols have their principal
        function in the formation of all Interior and Exterior cell
        membranes.

vi.   Lipoproteins (fat and protein) are important cellular
        constituents occurring both in the cell membrane and in
        the mitochondria and in cytoplasm. Also, lipoprotein
        serves as a means of transporting lipids in the blood.

vii.   Fats serve as carriers for the fat-soluble vitamins. The fat-
        soluble vitamins are, Vitamins A, D, E and K (Vitamins
        A, D, E and K).

3.5   Classification of Lipids

Bloor classified lipids as follows:

Simple Lipids

These are esters of fatty acids with various alcohols; example of simple
lipids include fats (esters of fatty acids with glycerol), waxes.

Compound Lipids

These are ester of fatty acids but containing in addition, alcohol and a
fatty acid. Examples of compound lipids include phospholipids, e.g.
(glycerophospholipids),   sphingophospholipids,   cerebrosides
(glycolipids) – compounds containing the fatty acids with carbohydrates,
containing nitrogen but no phosphoric acid. Other compound lipids
include sulpholipids, aminolipids, and lipoproteins.

Derived Lipids

These are substances derived from the hydrolysis of simple lipids and
compound lipids. The derived lipids include fatty acids, glycerol,
steroids, alcohols in addition to glycerol and sterols, fatty aldehydes and
ketone bodies; because glycerides (acylglycerols), cholesterol and
cholesteryl esters are unchanged, they are also termed neutral lipids.

3.6   Fatty Acids

Fatty acids are a group of aliphatic carboxylic (-COOH) acids, which
contain from 2 to 24 or more carbon atoms. Fatty acids are obtained
from the hydrolysis of fats. However, fatty acids can also occur in

natural fats and such fatty acids normally contain an even number of carbon atoms (i.e. from 2-carbon units) and are also straight-chain derivatives (aliphatic).

Fatty acids can either be saturated or unsaturated. We shall elaborate on this shortly. Fatty acids can also be straight-chain or branched. However, the most abundant types of fatty acid are saturated and unsaturated straight-chain fatty acids. Before we get to discuss how fatty acids are named (nomenclature), let us first look at saturated and unsaturated fatty acids.

Saturated Fatty Acids

These are fatty acids that do not contain any unsaturated bonds (i.e. having single bonds). The general formula for the saturated fatty acid is **CnH2n + 1COOH** – the first member of this group is acetic acid. Others will be discussed later.

Unsaturated Fatty Acids

These are fatty acids that contain one or more double bonds (unsaturated bonds). Unsaturated fatty acids with only one double bond are called monounsaturated fatty acids or monoethenoid acids. Those with more than one double bond are referred to as polyunsaturated fatty acids (PHFA) or polyethenoid acids

Essential Fatty Aacids

Three polyunsaturated fatty acids	Importance
Linoleic acid $\{C_{18} H_{32} O_2\}$	Play vital role in CNS peripheral nervous system
Linolenic acid $\{C_{18} H_{30} O_2\}$	Essential for some Spp of fish
Arachidonic acid $\{C20 H32O2\}$	Source of material for the biosynthesis of prostaglandins.

3.7   Digestion of Dietary Lipid

The bulk of dietary lipid is triglyceride (approx 90%) of animal or vegetable origin. In the gastrointestinal tract (GIT), a portion undergoes hydrolysis to constituent fatty acid and glycerol. Incompletely hydrolysis yields a measure of mono- and diglycerides in addition to the final product of the process. A group of "esterases called lipases" are of primary importance in the hydrolysis of triglycerides.

Gastric Digestion of Lipids

It has long been known that lipase is present in gastric juice, the optimal action of this enzyme is near neutrality and at the low pH level encountered in the stomach it is essentially inactive. Its significance is therefore uncertain, although some fatty acid appears to be liberated in the stomach. However, it has been suggested that gastric lipase may be more important in the infant since the gastric acidity is far lower in the infancy and since the normal lipid of the infant diet occurs in a highly emulsified state in milk.

Intestinal Digestion of Lipids

The major site of lipid digestion is the small intestine. In the duodenum, the bolus of the food encounters the bile in the pancreatic juices in the lower small intestine, the secretions of the intestinal mucousa are called *Succus entericus* which also participate in lipid digestion.

The role of the bile

Bile apparently contains no lipolytic enzymes. Its function in lipid digestion and absorption is to promote emulsification and solubilisation of lipids and this function is associated with the salt of bile acids. The bile and pancreatic juice are somewhat alkaline and serve in part to neutralise the acidic gastric chyme. In the approximately neutral environment of the duodenal lumen the bile acids, largely taurocholic and glycocholic acids, exist as anions, and and serve as detergent or emulsifying agents. In the presence of these detergents the churning effect of peristalsis results in a progressively finer and finer state of distribution of the dietary lipid in the continuous aqueous phase, facilitating lipolysis.

Since lipolysis (hydrolysis of lipids) involves participation of water and water-insoluble lipases, and since dietary lipids are essentially insoluble in water in water, hydrolysis occurs only at the interface between the lipid droplet and the aqueous phase. The rate of reaction is in part determined by the area of their interface, and the higher the degree of emulsification, the smaller the individual lipid droplets and the larger this area will be. The function of bile in lipid digestion is to promote contact between water-soluble and water-insoluble components of the lipolytic reaction.

3.8   Pancreatic Lipase

The flow of pancreatic juice, like the flow of bile, is regulated hormonally after the introduction of gastric chyme into the duodenum. A

precursor of lipase in the pancreatic juice becomes active in the intestinal lumen. The mechanism of activation of pancreatic lipase is not clear, but it has been suggested that a cofactor is needed for its activity. Pancreatic lipase acids best on fatty acid esters in the emulsified state. The degree of unsaturation and chain length has no significant effect on the rate of hydrolysis. Ca^{2+} has an accelerating effect on the enzyme, mainly because it forms insoluble soaps with liberated fatty acids.

3.9   Intestinal Absorption of Lipids

After ingestion of a fatty meal, the small intestine contains free fatty acid as their soaps together with a mixture of mono-, di-, and triglycerides well emulsified by the bile salts and the soaps themselves. A major portion of this mixture is absorbed across the wall of the small intestine such glycerol as is liberated is water soluble, and together with other water soluble nutrients is absorbed by the portal route. The fatty acids, on the other hand, are delivered to the organism predominantly via the intestinal lymph, where they appear in the form of triglycerides.

3.10   Digestion and Absorption of Fats

Fat digestion and absorption differ principally from the respective processes of digestion of carbohydrates and proteins, since fats are non-polar and are not miscible with water. The primary objective of lipid digestion is to arrange the lipid in a form that is water miscible and can be absorbed through the microvilli of the small intestine, which are covered by an aqueous layer. The sequence of event is the same in all animals:

i.   Lipolysis
ii.   Micellar solubilisation of the products of lipolysis
iii.   Uptake of the solubilised products by the intestinal mucosa
iv.   Resynthesis of triglyceride in the mucosal cells
v.   Secretion of triglycerides into the blood.

Micelles
Micelles are water-soluble aggregates of lipid molecules containing polar and non-polar groups. The molecules are grouped in the micelles in such a way that the polar groups are on the outside, in contact with the aqueous phase, while the non-polar parts form the inner lipid core of the micelles. The micelles produced in the lumen of the duodenum are very fine dispersion of lipids in water only 50-100 A in diameter, and carry the lipid digestion products (fatty acids, monoglycerides) to the mucosal cells of the small intestine where they are subsequently absorbed.

3.11   Factors Affecting Absorption of Lipids

- Differences in water solubility
- Protein interactions
- Micelle formation
- Enzyme specificity (with respect to triglyceride resynthesis)
- Liver dysfunction
- Extrahepatic biliary obstruction or biliary fistula
- Defect of the intestinal mucosa
- Absence of detergents (chiefly bile salts)

Note that: when bile is totally excluded from the intestinal tract due to liver dysfunction-it impedes lipid absorption. The bile salts' role in fat absorption is associated with their detergent properties. The bile acids do not enter the lymphatic circulation, rather confined to an enterohepatic circulation i.e. entering the portal blood from which they are removed by the liver and reinjected with the bile into the duodenum.

3.12   Body Lipids

In the normal mammal, 10 per cent or more of the body weight may be lipid, the bulk of which is **triglyceride.** This lipid is distributed in varying amounts in all organs as well as in certain depots of highly specialised connective tissues (the adipose tissue) in which a large fraction of the cytoplasm of the cell appears to be replaced by the droplets of the lipid. The large amount of lipid found in most animal tissues is in contrast to the situation in plants, where lipid is found in abundance only in seeds.

3.13   Blood Lipids

Normal blood plasma in the post absorptive state in man contains some 500mg of total lipid per 100ml of which about one-quarter is triglyceride. The increase in blood lipid is called LIPEMIA, and specifically that which transiently follows ingestion of fat is called ABSORPTIVE LIPEMIA

3.14   Lipid Transport and Storage

Fat absorbed from the diet and lipids synthesised by the liver and adipose tissue must be transported between the various tissues and organs for utilisation and storage. Since lipids are insoluble in water, the problem arises of how to transport them in an aqueous environment-the blood plasma. Lipids are transported from the mucosal cells of the intestine to various tissues by the circulation mainly as lipoproteins and

to a lesser extent as free fatty acids. The protein moiety of lipoproteins imparts water - soluble properties to lipids and allows their transport in the blood. The lipoprotein can be separated by a density-gradient procedure into different fractions.

The lipoproteins present in blood range from those of very low density to those of high density. The density increases as the proportion of protein in the lipoprotein increases and the lipid decreases. These protein-coated particles are called chylomicrons; chylomicrons contain the lightest lipoproteins. Lipoprotein transport lipid from the <u>intestine</u> as chylomicrons and from the <u>liver</u> as <u>Very Low Density Lipoprotein</u> (<u>VLDL</u>) to most tissues for oxidation and adipose tissues for storage.

3.15   Deposition of Fats in Tissues

The lipids are very rapidly removed from the blood by ADIPOSE TISSUE, LIVER and OTHER TISSUES. Adipose tissue is the most notable STORAGE SITE of fats in animals. Adipose tissue is mainly found under the skin and also around internal organs (heart, kidney). Adipose tissues help in the assimilation of carbohydrate and lipid and their intermediates for fat synthesis and storage. It also helps in the mobilisation of lipid as free fatty acids and to a more limited extent as glycerol. Both are influenced by hormones. The free fatty acids can be disposed of in the tissues in a variety of ways: (1) they may be completely oxidized to CO_2 and H_2O for release of energy (2) they may be esterified to form to reform triglycerides which may be released again into circulation or deposited in tissues for storage (3) a small proportion is transported in the blood complexed with albumin.

3.16   Biosynthesis of Fatty Acids

a)   Liver, adipose tissue and lactating mammary gland are the primary site of biosynthesis of fatty acids (f.as) and triglycerides. The main starting material for biosynthesis of f.as is acetyl-CoA derived from glucose, degraded fats and certain amino acids.

b)   Any substance capable of yielding acetyl-CoA is a potential source of C-atoms in the process of fatty acid synthesis (LIPOGENESIS).

c)   In monogastric, glucose is the primary substrate for lipogenesis and acetate in ruminant

Different Systems for Biosynthesis of Fatty Acids

There are at least **two** different systems for synthesis of f.as, both of which involve acetyl-CoA (the principal building block of fatty acids)

i. A Nonmitochondrial System-this system converts acetyl CoA to a long- chain f.as when supplemented with ATP, CO_2, MN^{2+}, TPNH.

ii A Mitochondrial System- this system utilises DPNH, TPNH, and ATP. This system appears to be mainly involved in elongation of f.as by addition of acetyl CoA to fatty acyl CoA compounds.

3.17 Ketone Bodies

After degration of a fatty acid, acetyl CoA is further oxidized in the citric acid cycle (CAC). If excess acetyl CoA is produced from – oxidation, some is converted to ketone bodies. These are acetoacetic acid, – Hydroxybutyric acid,, and acetone. All these products stem from acetoacetyl CoA, a normal intermediate in the oxidation of fatty acids.

The Synthesis and Utilisation of Ketone Bodies

Ketone bodies are two molecules, acetoacetate and -hydroxybutyrate. The term "ketone body" is historical: only acetoacetate is an actual ketone. Ketone bodies are synthesised in the liver from acetyl-CoA. The brain generally uses 60-70 –per cent of total body glucose requirements, and always requires some glucose for normal functioning. Under most conditions, glucose is essentially the sole energy source of the brain. The brain cannot use fatty acids, which cannot cross the blood-brain barrier; because animals cannot synthesise significant amounts of glucose from fatty acids, as glucose availability decreases, the brain is forced to use either amino acids or ketone bodies for fuel. Individuals eating diets extremely high in fat and low in carbohydrates, or starving, or suffering from a severe lack of insulin (Type I diabetes mellitus) therefore increase the synthesis and utilisation of ketone bodies.

Ketone body synthesis occurs normally under all conditions. However, the formation of ketone bodies increases dramatically during starvation. This seems to be due to a combination of factors. Prolonged low levels of insulin result in both increased fatty acid release from adipose tissue, and increased amounts of the enzymes required to synthesise and utilise ketone bodies. In addition, in the liver, increased demand for gluconeogenesis results in depletion of oxaloacetate, and therefore in decreased capacity for the TCA cycle. This causes a rise in the levels of acetyl-CoA, the substrate for ketone body production.
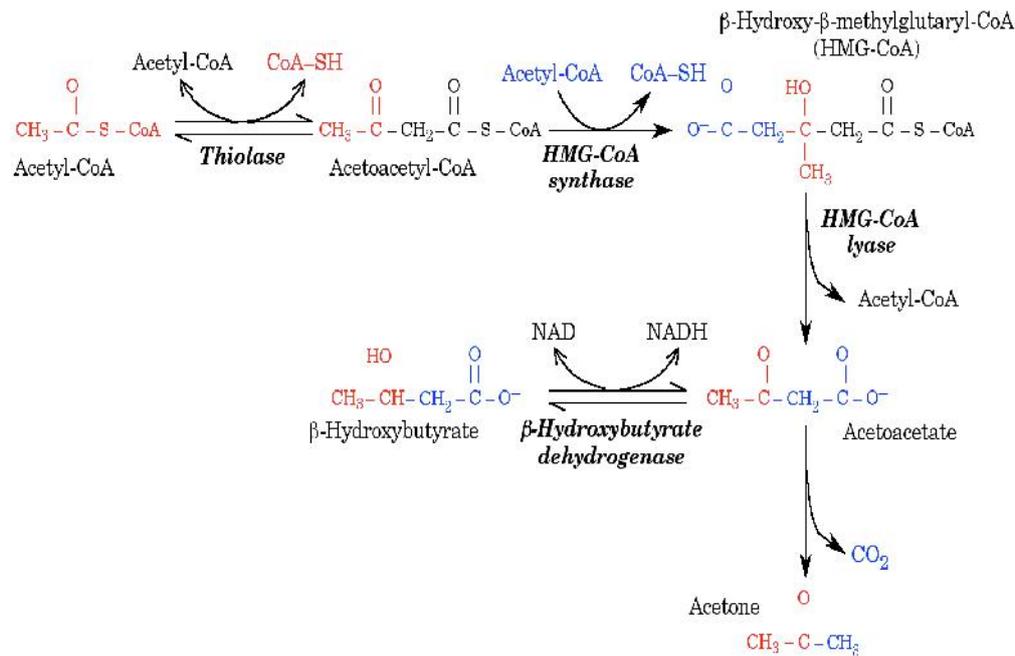
Fig.5.1

The first enzyme in the ketone body synthesis pathway is **thiolase** (the same enzyme that is responsible for the cleavage step in -oxidation). In ketone body biosynthesis, thiolase catalyses the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA. The next enzyme, **HMG-CoA synthase** adds a third acetyl CoA molecule, to form - hydroxy- -methylglutaryl-CoA (usually abbreviated HMGCoA). HMG-CoA is an important biosynthetic intermediate; however, in the mitochondria, it is only used for ketone body synthesis. The third enzyme, **HMGCoA lyase**, releases an acetyl-CoA from HMG-CoA to form acetoacetate. The final enzyme in ketone body synthesis, b**hydroxybutyrate dehydrogenase**, reduces acetoacetate to - - hydroxybutyrate. The -hydroxybutyrate dehydrogenase reaction has two functions: 1) it stores energy equivalent to an NADH in the ketone body for export to the tissues, and 2) it produces a more stable molecule. Acetoacetate is a -ketoacid, and like many such compounds may spontaneously decarboxylate. The product of the decarboxylation reaction, acetone, is a volatile waste product, and is largely excreted via the lungs.

Control of ketone body synthesis

Several factors influence ketone body production. Ketone body production only occurs during conditions of high circulating free fatty acids. One possible fate for the fatty acids is ketone body production, while another possible fate is conversion to triacylglycerol. However, because the glycerol required for triacylglycerol synthesis is derived from glycolysis, when glycolytic and gluconeogenic substrates are

limiting the liver will make primarily ketone bodies. Since oxidation of acetyl CoA via the citric acid cycle depends on a source of oxaloacetate and thus may arise by carboxylation of pyruvate derived from glycolysis. Thus, f.as oxidation also depends on carbohydrate metabolism.

4.0 CONCLUSION

In this unit, you learnt that lipids are organic compounds found in plant and animal tissue, and are oily/greasy substances. They are also soluble in organic solvents such as benzene, ether or chloroform, but only sparingly soluble in water.

5.0 SUMMARY

Lipids are biological substances that are soluble in organic solvents. Apart from being structural components of biological membrane, they also play a major role in the nutrition of the animal. The lipids are very rapidly removed from the blood by adipose tissue, liver and other tissues. Adipose tissue is the most notable storage site of fats in animals. Adipose tissue is mainly found under the skin and also around internal organs (heart, kidney).

Adipose tissues help in the assimilation of carbohydrate and lipid and their intermediates for fat synthesis and storage. It also helps in the mobilisation of lipid as free fatty acids and to a more limited extent as glycerol. Both are influenced by hormones. The free fatty acids can be disposed of in the tissues in a variety of ways: (1) they may be completely oxidised to CO_2 and H_2O for release of energy (2) they may be esterified to form to reform triglycerides which may be released again into circulation or deposited in tissues for storage (3) a small proportion is transported in the blood complexed with albumin.

6.0 TUTOR-MARKED ASSIGNMENT

1.   Distinguish between gastric and intestinal digestion of lipids.
2.   What is the role of the bile in lipid digestion?
3.   How would classify lipids?
4.   Name five factors affecting the absorption of lipids.
5   Write short notes on the following:
a).   Ketone bodies
b).   Deposition of fat in tissues
c).   Lipid transport and storage
d).   Control of ketone body synthesis
e).   Deposition of fats in tissues
f).   Biosynthesis of fatty acids.

7.0   REFERENCES/FURTHER READING

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