CARBOHYDRATES II

Eric Mbindo Njunju

Bsc; Msc

DIGESTION OF CARBOHYDRATES

In the diet, carbohydrates are present as complex polysaccharides (starch, glycogen), and to a minor extent, as disaccharides (sucrose and lactose).

Hydrolyzed to monosaccharide units in GIT

(Cooking makes the digestion process easier)

Process of digestion starts in mouth by the salivary alpha-amylase.

Time available for digestion in the mouth is limited, because the gastric hydrochloric acid will inhibit the action of salivary amylase.

In the pancreatic juice another alpha-amylase is available, which will hydrolyze the alpha-1,4 glycosidic linkages randomly, so as to produce smaller subunits like maltose, isomaltose, dextrins and branched or unbranched oligosaccharides.

Cells of brush border of intestine contain the enzymes, sucrase, maltase, isomaltase and lactase.

Hydrolyze the corresponding disaccharides into component monosaccharides, which are then absorbed.

Clinical Application- Lactose Intolerance

- Lactase hydrolyzes lactose to glucose and galactose

Lactase is present in the brush border of enterocytes

- Deficiency of lactase leads to lactose intolerance. In this condition, lactose accumulates in the gut. Irritant diarrhea and flatulence are seen.
- The causes may be congenital or acquired. As age advances, lactase activity decreases and secondary lactose intolerance occurs. Acquired lactose intolerance can also occur when there is a sudden change to a milk based diet. Lactase is an inducible enzyme. If milk is withdrawn temporarily, diarrhea will be limited. Curd is also an effective treatment, because the lactobacilli present in curd contains the enzyme lactase. Lactase is abundantly seen in yeast, which could also be used in treatment.

ABSORPTION OF CARBOHYDRATES

- Only monosaccharides are absorbed by the intestine.
- Absorption rate is maximum for galactose; moderate for glucose; and minimum for fructose.

Absorption of Glucose

- Glucose has specific transporters, which are transmembrane proteins.

Absorption of other Monosaccharides

- Glucose and galactose absorbed by the same transporter, SGluT.
- An energy dependent process, against a concentration gradient, and therefore absorption is almost complete from the intestine.
- Other monosaccharides absorbed by carrier mediated facilitated transport. Therefore, absorption is not complete, and the remaining molecules in the intestine will be fermented by bacteria.

Clinical Importance of Glucose

1. Glucose-preferred source of energy for most of the body tissues. Brain cells derive the energy mainly from glucose.

2. Glucose metabolism is deranged-Life threatening conditions may occur. A minimum amount of glucose is always required for normal functioning.

3. Normal fasting plasma glucose level is 70 to 110 mg/dL**.** After a heavy carbohydrate meal, in a normal person, level is below 150 mg/dL.

GLYCOLYSIS (EMBDEN-MEYERHOF-PARNAS PATHWAY)

In glycolytic pathway, glucose is converted to pyruvate (aerobic condition) or lactate (anaerobic condition), along with production of a small quantity of energy.

Glycolysis is derived from the Greek words, glykys= sweet; and lysis = splitting.

Site of reactions: All the reaction steps take place in the cytoplasm.

Significance of Glycolysis Pathway

1. Only pathway that is taking place in all the cells of the body.

2. Only source of energy in erythrocytes.

3. In strenuous exercise, when muscle tissue lacks enough oxygen, anaerobic glycolysis forms the major source of energy for muscles.

4. May be considered as the preliminary step before complete oxidation.

5. Provides carbon skeletons for synthesis of non-essential amino acids as well as glycerol part of fat.

6. Most of the reactions are reversible, which are also used for gluconeogenesis.

Glucose Entry into Cells

Glucose transporter-4 (GluT4) transports glucose from the extracellular fluid to muscle cells and adipocytes. This translocase is under the influence of insulin.

In diabetes mellitus, insulin deficiency hinders the entry of glucose into the peripheral cells. But GluT2 is the transporter in liver cells; it is not under the control of insulin.

Steps of Glycolytic Pathway

- *Step 1 of Glycolysis*

i. Glucose is phosphorylated to glucose-6-phosphate.

ii. The enzyme is Hexokinase (HK), which splits the ATP into ADP, and the Pi is added on to the glucose.

- The energy released by the hydrolysis of ATP is utilized for the forward reaction.

iii. Hexokinase is a key glycolytic enzyme. The kinase reaction is irreversible. But this irreversibility is circumvented by another enzyme glucose-6-phosphatase (see gluconeogenesis).

iv. Hexokinase and glucokinase may be considered as iso-enzymes; their properties are compared in Table 9.2.

Glucokinase is under the influence of insulin; but hexokinase is not. Hexokinase is present in most tissues. Glucokinase with a high Km for glucose is present in liver and beta cells. Glucokinase is induced by insulin.

v. The metabolic fates of Glucose-6-phosphate are shown in Figure. The phosphorylation of glucose traps it within the cells. Once phosphorylated, glucose-6- phosphate is trapped within the cell and has to be metabolized.

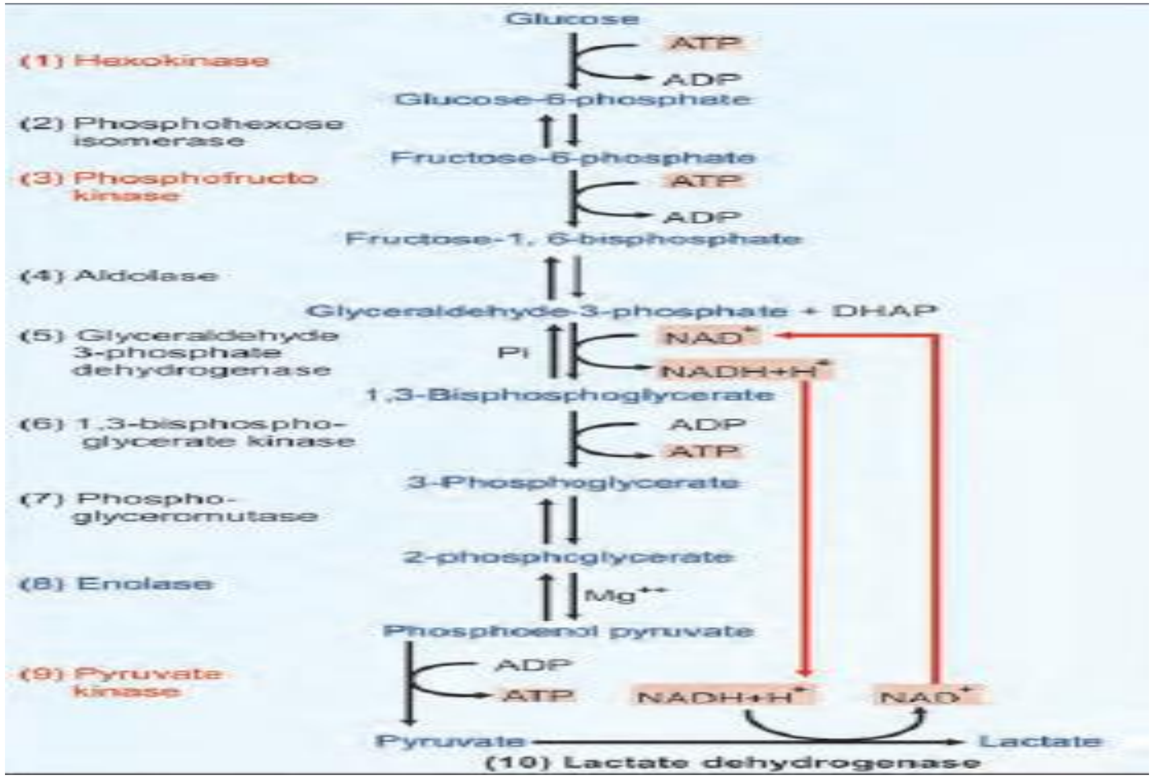

Fig: Summary of glycolysis (Embden-Meyerhof - Parnas pathway). Steps 1, 3 and 9 are key enzymes; these reactions are irreversible. Steps 6 and 9 produce energy. Steps 5 and 10 are coupled for regeneration of NAD+

Step 2 of Glycolysis

Glucose-6-phosphate is isomerized to fructose-6-phosphate by phosphohexose isomerase. This is readily reversible.

This isomerisation of aldose to ketose involves the opening of the glucopyranose ring of glucose-6-phosphate to a linear structure which then changes to the furanose ring structure of fructose-6-phosphate.

- *Step 3 of Glycolysis*

i. Fructose-6-phosphate is further phosphorylated to fructose1,6-bisphosphate. The enzyme is phosphofructokinase.

ii. Phosphofructokinase (PFK) is the rate limiting enzyme of glycolysis. It is an allosterically regulated enzyme.

The enzyme catalyzes the second phosphorylation step of glycolysis using a second molecule of ATP.

- PFK is an allosteric, inducible, regulatory enzyme. It is an important key enzyme of this pathway.

- Glycolysis takes place in cytoplasm.
- Pyruvate is generated in cytoplasm.
- Pyruvate is then transported into mitochondria by a pyruvate transporter

Amino Sugars

- Amino groups may be substituted for hydroxyl groups of sugars to give rise to amino sugars. Generally, the amino group is added to the second carbon atom of hexoses.
- Amino sugars will not show reducing property. They will not produce osazones.

- Glucosamine is seen in hyaluronic acid, heparin and blood group substances.
- Galactosamine is present in chondroitin of cartilage, bone and tendons.
- Mannosamine is a constituent of glycoproteins.

The amino group in the sugar may be further acetylated to produce N-acetylated sugars such as N-acetyl-glucosamine (GluNac), N-acetylgalactosamine (GalNac), etc. which are important constituents of glycoproteins, mucopolysaccharides and cell membrane antigens.

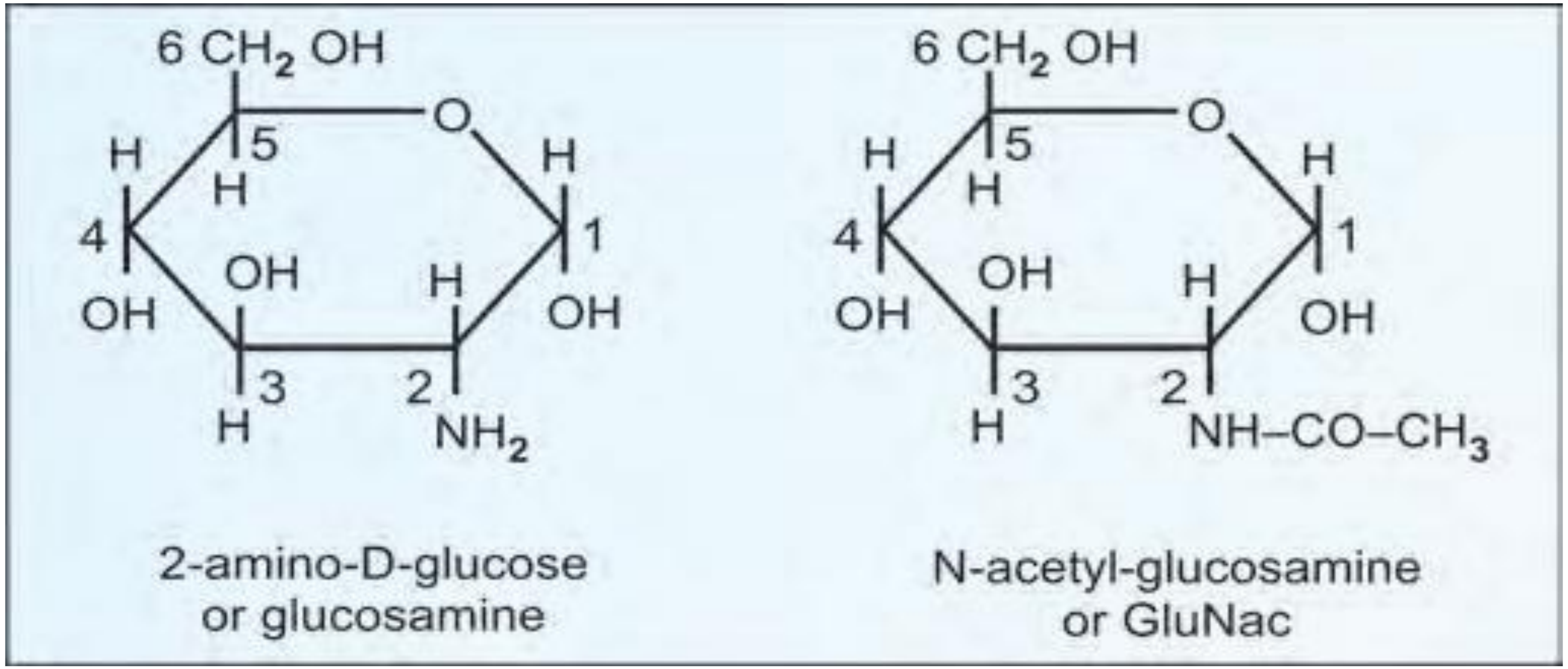

Fig: Amino sugars

Deoxy Sugars

i. Oxygen of the hydroxyl group may be removed to form deoxy sugars. Some biologically important deoxy sugars are shown in Figure.

ii. Deoxy sugars will not reduce and will not form osazones. L-fucose is present in blood group antigens and many other glycoproteins.

iii. Deoxyribose (Fig.) is an important part of nucleic acid. Feulgen staining is specific for 2-deoxy sugars (and DNA) in tissues; this is based on the reaction of 2-deoxy sugars with Schiff's reagent (Dye Fuchsine is decolorized by sulfurous acid).

Pentoses

i. Sugars containing 5 carbon atoms.

ii. Ribose (Fig.) is a constituent of RNA. Ribose is also seen in co-enzymes such as ATP and NAD.

Deoxyribose is seen in DNA (Fig.)

iii. Ribulose is an intermediate of HMP shunt pathway.

Arabinose is present in cherries and seen in glycoproteins of the body. The name arabinose is derived as it was originally isolated from gum arabic.

iv. Xylose is seen in proteoglycans. Xylulose is an intermediate of uronic acid pathway.

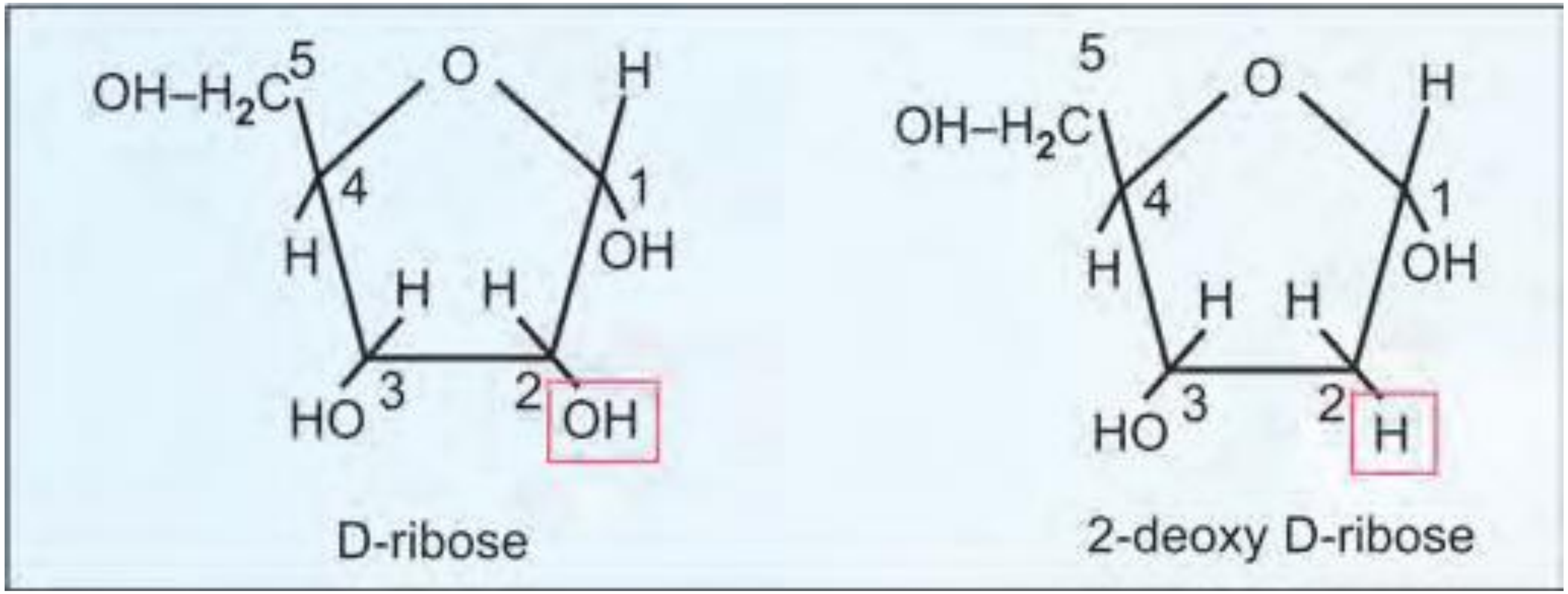

Fig: Sugars of nucleic acids

POLYSACCHARIDES

Polymerized products of many monosaccharide units.

1. Homoglycans are composed of single kind of monosaccharides, e.g. starch, glycogen and cellulose.

2. Heteroglycans are composed of two or more different monosaccharides, e.g. hyaluronic acid, chondroitin sulfate.

Starch

Structure of starch

- Reserve carbohydrate of plant kingdom.

Sources: Potatoes, tapioca, cereals (rice, wheat) and other food grains.

Composed of amylose and amylopectin.

- When treated with boiling water, 10-20% is solubilized; this part is called amylose. Amylose is made up of glucose units with alpha-1,4 glycosidic linkages (Fig.) to form an unbranched long chain with a molecular weight 400,000 D or more.

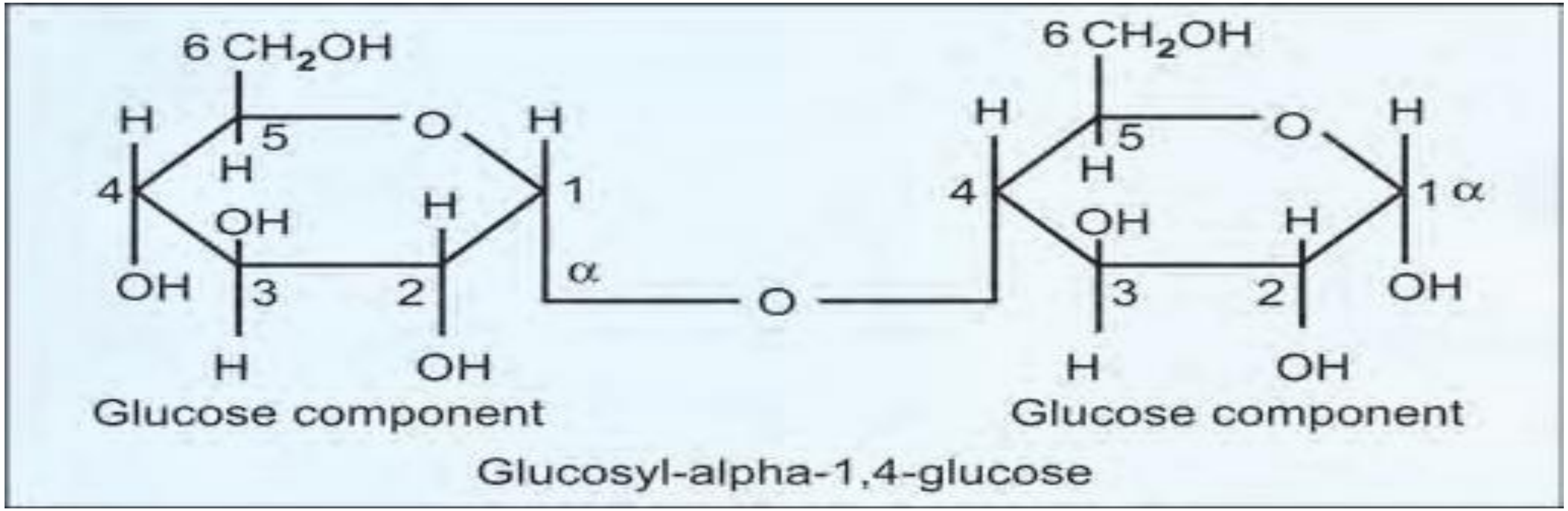

Fig: Showing the alpha-1,4 glycosidic linkages in maltose

Insoluble part absorbs water and forms paste like gel called amylopectin. Amylopectin is also made up of glucose units, but is highly branched with molecular weight more than 1 million.

The branching points are made by alpha-1,6 linkage (similar to isomaltose, Fig.)

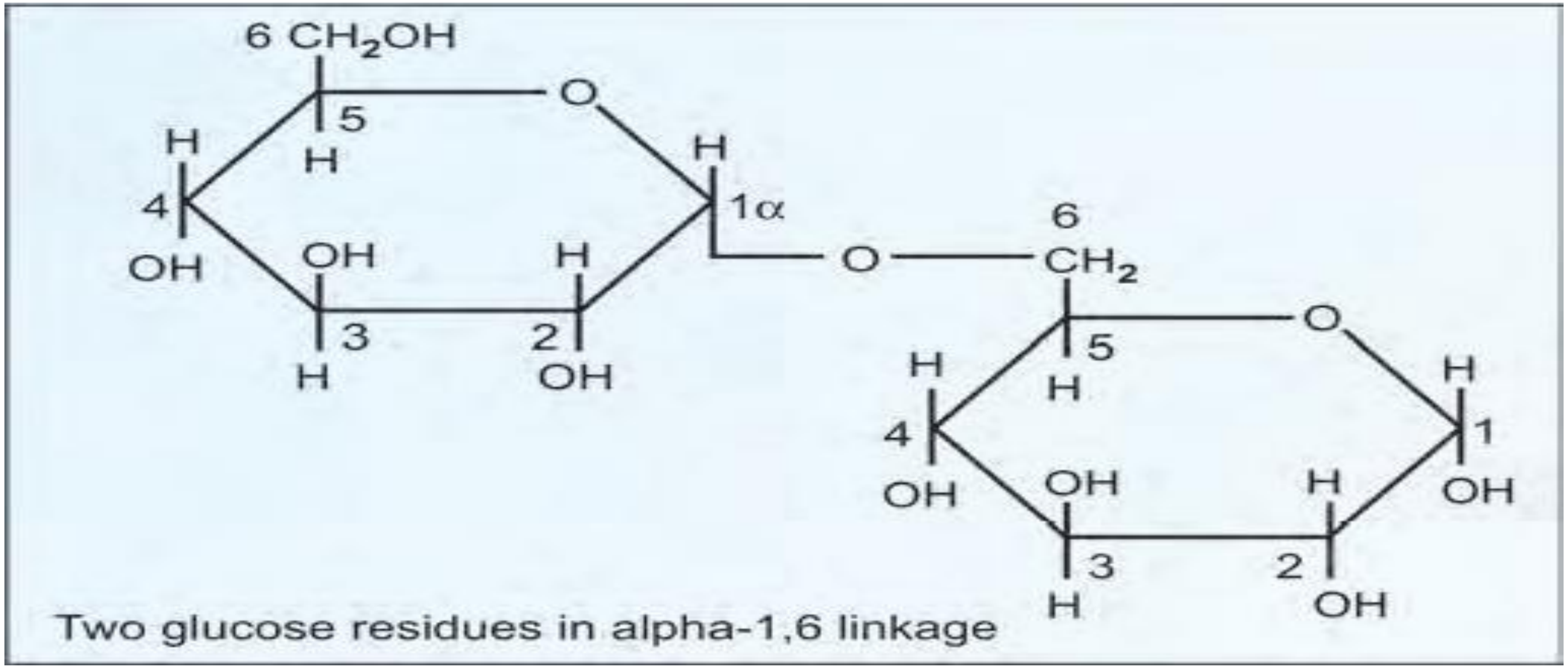

Fig: showing the alpha-1, 6 linkage is Isomaltose

- *Action of Amylases on Starch*

i. Salivary amylase and pancreatic amylase are alpha amylases, which act at random on alpha-1,4 glycosidic bonds to split starch into smaller units (dextrins), and finally to alpha-maltose.

ii. Beta-amylases are of plant origin (almond, germinating seeds, etc.) which split starch to form beta-maltose. They act on amylose to split maltose units consecutively

When beta-amylase acts on amylopectin, maltose units are liberated from the ends of the branches of amylopectin, until the action of enzyme is blocked at the 1,6-glycosidic linkage. The action of beta-amylase stops at branching points, leaving a large molecule, called limit dextrin or residual dextrin.

Glycogen

- Reserve carbohydrate in animals.
- Stored in liver and muscle.
- About 5% of weight of liver is made up by glycogen.
- Excess carbohydrates are deposited as glycogen.

Glucose units joined by alpha-1,4 links in straight chains.

It also has alpha-1,6 glycosidic linkages at the branching points.

- Molecular weight of glycogen is about 5 million. Innermost core of glycogen contains a primer protein, Glycogenin. Glycogen is more branched and more compact than amylopectin.

Cellulose

Supporting tissues of plants.

Constitutes 99% of cotton, 50% of wood and is the most abundant organic material in nature.

- Glucose units combined with beta- 1,4 linkages. A straight line structure, with no branching points. Molecular weight is in the order of 2 to 5 million.
- Beta-1,4 bridges are hydrolyzed by the enzyme cellobiase. But this enzyme is absent in animal and human digestive system, and hence cellulose cannot be digested.
- Herbivorous animals have large cecum, which harbor bacteria. The bacteria can hydrolyze cellulose, and the glucose produced is utilized by the animal. White ants (termites) also digest cellulose with the help of intestinal bacteria.

- Cellulose has a variety of commercial applications, as it is the starting material to produce fibers, celluloids, nitrocellulose and plastics.

Inulin

- A long chain homoglycan composed of D-fructose units with repeating beta-1,2 linkages. Reserve carbohydrate present in various bulbs and tubers, such as chicory, dahlia, dandelion, onion, garlic. Clinically used to find renal clearance value and glomerular filtration rate.
- A polysaccharide (carbohydrate) made up of fructose units. Used for renal function studies.

Dextrans

- Highly branched homopolymers of glucose units with 1-6, 1-4 and 1-3 linkages. Produced by micro-organisms. Molecular weight 1 million to 4 millions. Since they will not easily go out of vascular compartment, they are used for intravenous infusion as plasma volume expander for treatment of hypovolemic shock. It may be noted that dextrans are different from previously described dextrins
- D-glucose is otherwise called Dextrose, a term often used in bed-side medicine, e.g. dextrose drip. Dextrin is the partially digested product of starch. Dextran is high molecular weight carbohydrate, synthesized by bacteria.

Chitin

- Present in exoskeletons of crustacea and insects.
- Composed of units of N-acetyl-glucosamine with beta-1,4 glycosidic linkages

HETEROGLYCANS

Polysaccharides containing more than one type of sugar residues.

Examples are:

Agar

i. Prepared from sea weeds. Contains galactose, glucose and other sugars.

ii. Dissolved in water at 100^0C, which upon cooling sets into a gel. Agar cannot be digested by bacteria and hence used widely as a supporting agent to culture bacterial colonies. Agar is used as a supporting medium for immunodiffusion and immunoelectrophoresis.

iii. Agarose is made up of galactose combined with 3,6-anhydrogalactose units; it is used as matrix for electrophoresis

MUCOPOLYSACCHARIDES [GLYCOSAMINO GLYCANS, (GAG)] Heteropolysaccharides, containing uronic acid and amino sugars. Acetylated amino groups, sulfate and carboxyl groups are also generally present. Because of the presence of these charged groups, they attract water molecules and so they produce viscous solutions. Mucopolysaccharides in combination with proteins form mucoproteins. Examples of mucopolysaccharides are hyaluronic acid, heparin, chondroitin sulfate, dermatan sulfate and keratan sulfate.

- Mucopolysaccharides are excreted in urine in abnormal amounts in the group of lysosomal storage disorders known as *mucopolysaccharidoses.* They can be detected by 2D gel electrophoresis techniques; some mucopolysaccharides can also be detected by simple urine screening tests.

Hyaluronic acid

- Present in connective tissues, tendons, synovial fluid and vitreous humor. Serves as a lubricant in joint cavities.
- Composed of repeating units of N-Acetyl-glucosamine→ beta-1, 4-Glucuronic acid → beta-1-3-N-Acetyl glucosamine and so on.

Heparin

- i. An anticoagulant widely used when taking blood *in vitro* for clinical studies. Used *in vivo* in suspected thromboembolic conditions to prevent intravascular coagulation.
- Activates antithrombin III, which in turn inactivates thrombin, factor X and factor IX.
- ii. Present in liver, lungs, spleen and monocytes. Commercial preparation of from animal lung tissues.
- iii. It contains repeating units of sulphated glucosamine → alpha-1, 4-L-iduronic acid → and so on (Fig).

Idose is the 5th epimer of glucose. Iduronic acid is the oxidized form of idose. Sulfated heparin or heparin sulfate is also present in tissues.

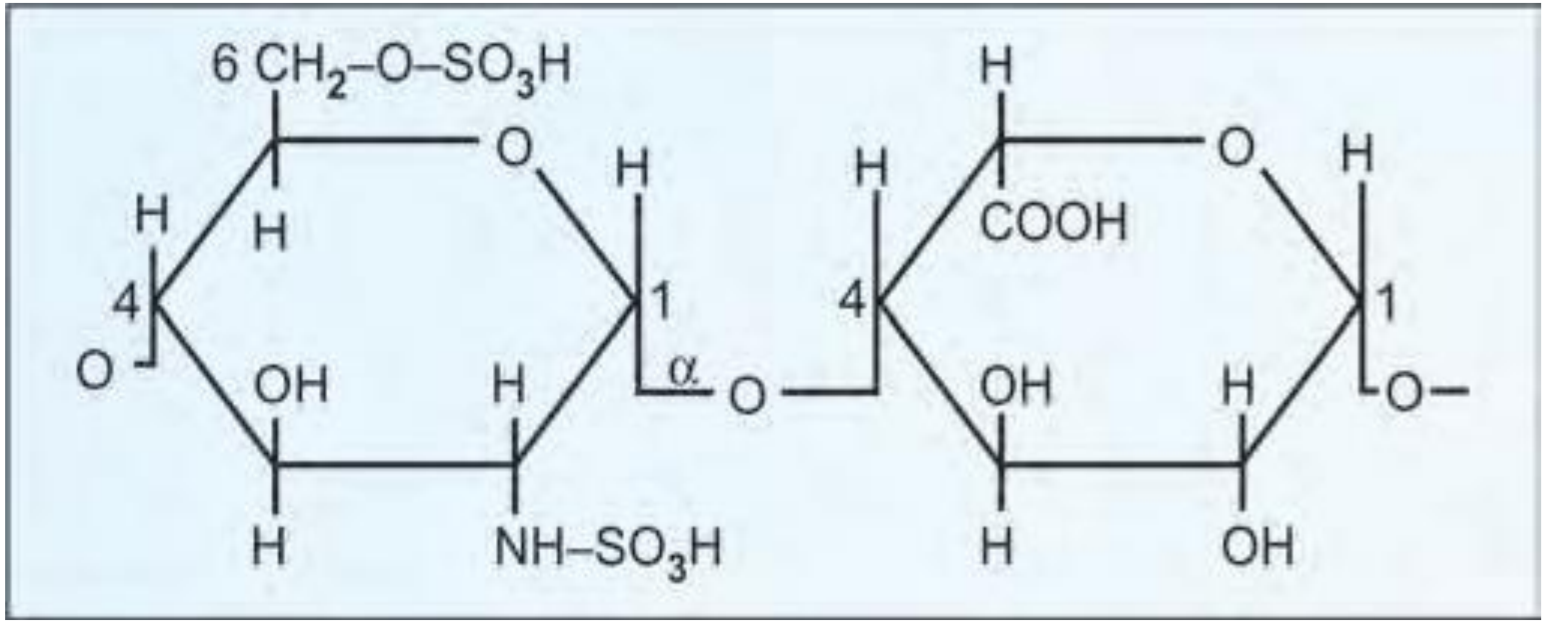

Fig: Sulfated glucosamine-alpha-1, 4-iduronic acid. Repeating units in heparin

Chondroitin Sulfate

- Present in ground substance of connective tissues (viscosity and plasticity) widely distributed in cartilage, bone, tendons, cornea and skin.
- Composed of repeating units of glucuronic acid→ beta-1,3-N-acetyl galactosamine sulfate → beta-1, 4 and so on

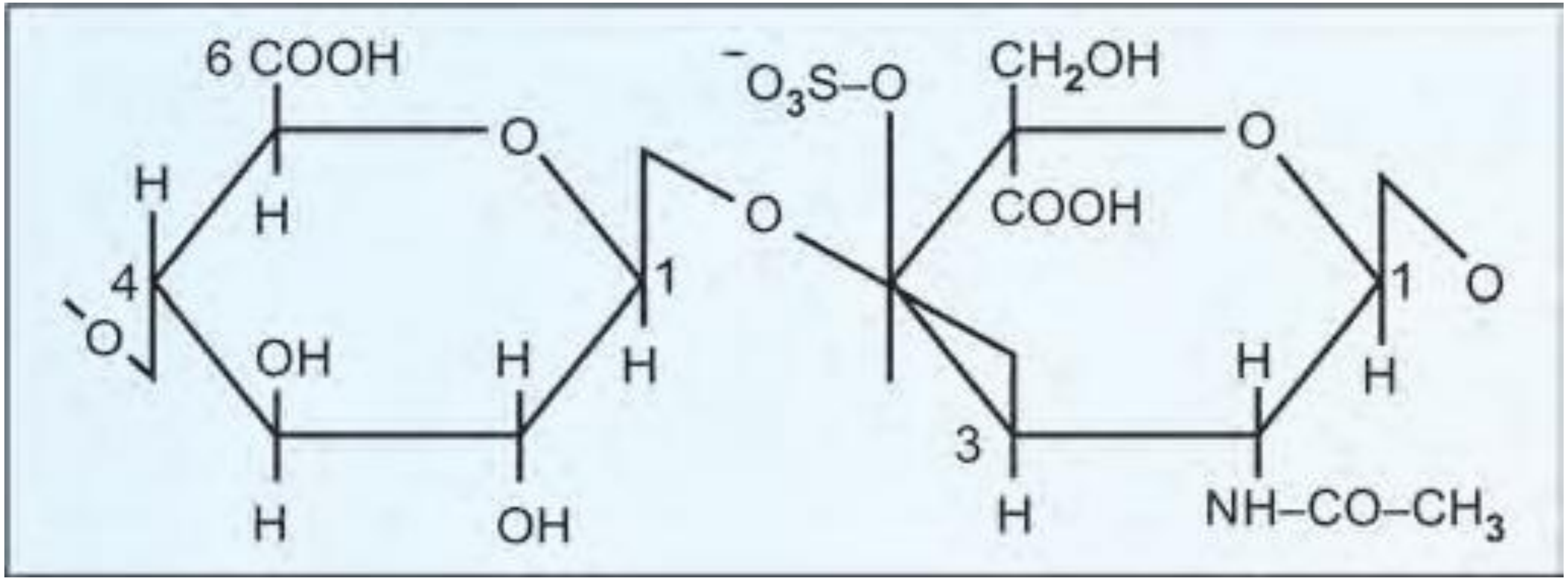

Fig: D-glucuronic acid-beta-1, 3-N-acetyl galactosamine-4 sulfate (units of chondroitin sulfate)

Keratan Sulfate

- Only GAG, which does not contain any uronic acid.
- Repeating units are galactose and N-acetyl glucosamine in beta linkage.
- Found in cornea and tendons.

Dermatan Sulfate

- Contains L-iduronic acid and N-acetyl galactosamine in beta-1, 3 linkages.
- Found in skin, blood vessels and heart valves

Polysaccharide	Repeating units
Homoglycans	
Inulin	D-fructose, beta-1,2 linkages
Dextran	Glucose, 1-6, 1-4, 1-3 linkages
Chitin	N-acetyl glucosamine; beta 1-4 links
Heteroglycans	
Agar	Galactose, glucose
Agarose	Galactose, anhydrogalactose
Hyaluronic acid	N-acetyl glucosamine, glucuronic acid
Heparin	Sulphated glucosamine, L-iduronic acid
Chondroitin S	Glucuronic acid, Nacetyl galactosamine
Keratan S	Galactose, N-acetyl glucosamine
Dermatan S	L-iduronic acid, N-acetyl galactosamine

Table: Repeating units in polysaccharides

GLYCOPROTEINS AND MUCOPROTEINS

- Carbohydrate chains attached to a polypeptide chain-proteoglycan.
- Carbohydrate content less than 10%-glycoprotein.
- Carbohydrate content more than 10%-mucoprotein.
- Seen in almost all tissues and cell membranes.

- About 5% of the weight of the cell membrane is carbohydrates Carbohydrate groups cover the entire surface of the cell membrane, (glycocalyx). Functions include their role as enzymes, hormones, transport proteins, structural proteins and receptors.

Glycophorin: Major membrane glycoprotein of erythrocytes.

Protein is transmembrane (spans the whole thickness of the membrane), Polypeptide chain is seen both inside and outside the membrane. Carbohydrate chains are attached to the amino terminal portion, outside the cell surface.

The oligosaccharide chains of glycoproteins are composed of varying numbers of the following carbohydrate residue: Glucose (Glu); mannose (Man); galactose (Gal); N-acetyl glucosamine (GluNAc); N-acetyl galactosamine (GalNAc); arabinose (Ara); Xylose (Xyl); L-fucose (Fuc) (fig.) and N-acetyl neuraminic acid (NANA).

Fig: Deoxy sugars

- Carbohydrate group is attached to proteins either as O-glycosidic linkages or as N-glycosidic linkages. The O-glycosidic linkage is GalNAc to serine or threonine residues of usual protein; however, galactose is added to hydroxylysine residues of collagen. The N-glycosidic linkages are made by addition of carbohydrate group to nitrogen atom of asparagine or glutamine residues of proteins. The oligosaccharides attached to proteins may alter physical properties such as size, shape, solubility, or stability, may effect folding, and/or may have biological roles.

Common Sugar Substitutes

Acesulfame-Potassium (Ace K):

- Made from aceto acetic acid.
- 200 times sweeter than sugar; but calorie content is negligible.
- It is present in artificial sweeteners, carbonated drinks, pharmaceutical products.
- It has a slightly bitter aftertaste. Unlike aspartame, acesulfame
- K is stable under heat, allowing it to be used in baking, or in products that require a long shelf life.

Aspartame

- Made from aspartic acid and phenyl alanine, both are amino acids. (Aspartame is the methyl ester of a phenylalanine/aspartic acid dipeptide). 200 times more sweet than sugar.
- Calorie content is 4 kcal per gram
- Under strongly acidic or alkaline conditions, aspartame may generate methanol by hydrolysis.
- Aspartame is immediately metabolized to phenylalanine, aspartic acid and methanol. Aspartame is not suitable for people with phenyl ketonuria.

Saccharin

- Made from anthranilic acid.
- 300 times sweeter than sugar.
- Calorie content is nil.

Sucralose

- Made from sucrose or table sugar.
- Approximately 600 times as sweet as sucrose (table sugar)
- Twice as sweet as saccharin
- 3.3 times as sweet as aspartame.
- Calorie content is nil.

Sodium cyclamate (Sodium cyclohexyl sulfamate)

- 30–50 times sweeter than sugar.
- Less expensive than most sweeteners and is stable under heating. The 10:1 cyclamate:saccharin mixture is found to increase the incidence of bladder cancer in rats
- Its sale is banned in the United States
- However, Cyclamate is approved as a sweetener in over 55 countries

Saccharin (Benzoic sulfimide):

- An artificial sweetener, but has an unpleasant bitter or metallic after taste, especially at high concentrations.
- Sweeten products such as drinks, candies, medicines, and toothpaste.
- Although saccharin has no food energy, it can trigger the release of insulin in humans and rats, apparently as a result of its taste.

Erythritol:

- Natural sugar alcohol.
- Occurs naturally in fruits and fermented foods.
- 60–70% as sweet as table sugar yet it is almost non-caloric,
- Does not affect blood sugar, does not cause tooth decay.
- Absorbed, and then for the most part excreted unchanged in the urine.

Xylitol

- Found in the fibers of many fruits and vegetables, including various berries, corn husks, oats, and mushrooms.
- Roughly as sweet as sucrose with only two-thirds the food energy.
- A safe sweetener for people with diabetes that would not impact insulin levels. Sugar substitutes can be used in the initial phase of a dieting plan for an obese child.

Dietary Fiber

- Contributed by the unavailable carbohydrates in the diet.
- Several different types have been found in different types of food items.
- Contribute the bulk and assist in normal bowel

Bacterial Cell Wall

- Major constituents of prokaryotic (bacterial) cells are heteropolysaccharides
- Consisting of repeating units of N-acetyl muramic acid (NAM) and N-acetyl glucosamine (NAG).
- Provides mechanical strength.
- Synthesis of this complex polysaccharide is blocked by penicillin. This inhibition is responsible for the bactericidal action of penicillin.