

# CARBOHYDRATES

**Definition:** Carbohydrates are defined chemically as aldehyde or ketone derivatives of the higher polyhydric alcohols, or compounds which yield these derivatives on hydrolysis.

## CLASSIFICATION

Carbohydrates are divided into **four** major groups' monosaccharides, disaccharides, oligosaccharides and polysaccharides.

**1. Monosaccharides:** (also called 'simple' sugars) are those which cannot be hydrolysed further into simpler forms.

**General formula :**  $C_nH_{2n}O_n$

They can be subdivided further:

**(a) Depending upon the number of carbon atoms** they possess, as trioses, tetroses, pentoses, hexoses, etc.

**(b) Depending upon whether aldehyde ( $-CHO$ ) or ketone ( $-CO$ ) groups** are present as aldoses or ketoses.

**General formula**

**Aldosugars**

**Ketosugars**

• **Trioses**

Glyceraldehyde

Dihydroxyacetone ( $C_3H_6O_3$ )

• **Tetroses**

Erythrose

Erythrulose ( $C_4H_8O_4$ )

• **Pentoses**

Ribose

Ribulose ( $C_5H_{10}O_5$ )

• **Hexoses**

Glucose

Fructose ( $C_6H_{12}O_6$ )

**2. Disaccharides:** Those sugars which yield two molecules of the same or different molecules of monosaccharide on hydrolysis. **General formula :**  $C_n(H_2O)_{n-1}$  or  $C_{12}H_{22}O_{11}$

**Examples**

• **Maltose** yields 2 molecules of glucose on hydrolysis.

• **Lactose** yields one molecule of glucose and one molecule of galactose on hydrolysis.

• **Sucrose** yields one molecule of glucose and one molecule of fructose on hydrolysis.

• **Lactulose** a ketodisaccharide

**3. Oligosaccharides:** Those sugars which yield 3 to 10 monosaccharide units on hydrolysis, e.g. Maltotriose.

**4. Polysaccharides (Glycans):** Those sugars which yield more than ten molecules of monosaccharides on hydrolysis.

**General formula:**  $(C_6H_{10}O_5)_n$

Polysaccharides are further divided into **two** groups:

**a. Homopolysaccharides (homoglycans):** Polymer of same monosaccharide units. **Examples** Starch, glycogen, inulin, cellulose, dextrans, dextrans.

**b. Heteropolysaccharides (heteroglycans):** Polymer of different monosaccharide units or their derivatives.

**Example**—Mucopolysaccharides (glycosaminoglycans)

## Biomedical Importance of Carbohydrates

• Chief source of energy.

• Constituents of compound lipids and conjugated proteins.

• Degradation products act as "promoters" or 'catalysts'.

• Certain carbohydrate derivatives are used as drugs like cardiac glycosides/antibiotics.

• Lactose principal sugar of milk—in lactating mammary gland.

• Degradation products utilised for synthesis of other substances such as fatty acids, cholesterol, amino acid, etc.

- Constituents of mucopolysaccharides which form the ground substance of mesenchymal tissues.
- Inherited deficiency of certain enzymes in metabolic pathways of different carbohydrates can cause diseases, e.g. galactosemia, glycogen storage diseases (GSDs), lactose intolerance, etc.
- Derangement of glucose metabolism is seen in diabetes mellitus.

### General Properties in Reference to Glucose

**Asymmetric carbon:** A carbon atom to which four different atoms or groups of atoms are attached is said to be asymmetric

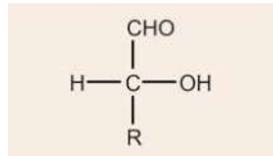


Fig. 3.1: Asymmetric carbon

**Van't Hoff's rule of 'n':** The number of possible isomers of any given compound depends upon the number of asymmetric carbon atoms the molecule possesses.

According to **Van't Hoff's rule of 'n'**;  $2^n$  equals the possible isomers of that compound, where,  $n =$  represents the number of asymmetric carbon atoms in a compound.

**Stereoisomerism:** The presence of asymmetric carbon atoms in a compound gives rise to the formation of isomers of that compound. Such compounds which are identical in composition and differs only in spatial configuration are called **stereoisomers**.

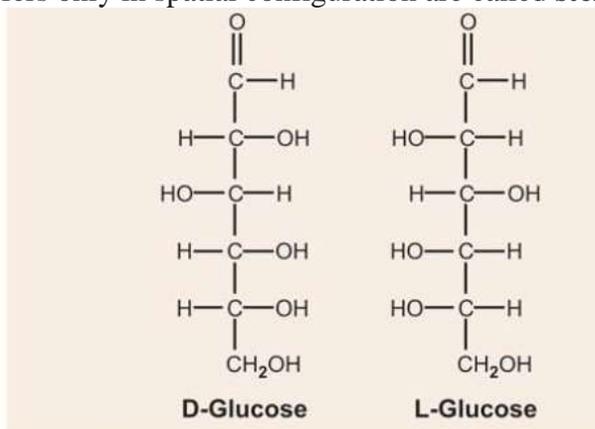


Fig. 3.2: Stereoisomers of glucose

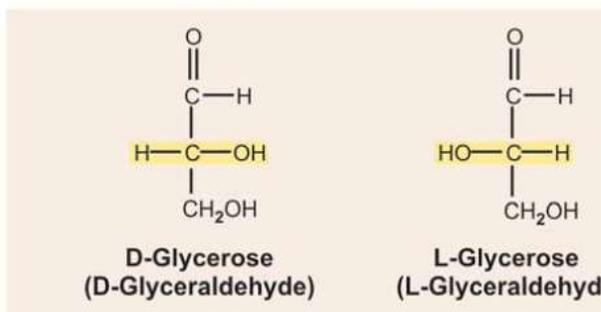


Fig. 3.3: D- and L-series

Enantiomers Two such isomers of glucose—D-Glucose and L-Glucose are **mirror image of each other** (Fig. 3.2).

**D-Series and L-Series:** The orientation of the H and OH groups around the carbon atom just adjacent to the terminal primary alcohol carbon, e.g. C-atom 5 in glucose determines the series. **When the – OH group on this carbon is on the right, it belongs to D-series, when the – OH group is on the left, it is a member of L-series.**

Most of the monosaccharides occurring in mammals are D-sugars, and the enzymes responsible for their metabolism are specific for this configuration.

**Optical activity:** Presence of asymmetric carbon atoms also confers optical activity on the compound. When a beam of *plane-polarised light* is passed through a solution exhibiting optical activity, it will be rotated to the right or left in accordance with the type of compound, i.e. the *optical isomers* or *enantiomorphs*; when it is rotated to right, the compound is called • **Dextrorotatory (D or + sign)**, when rotated to left, the compound is called • **Laevorotatory (L or – sign)**.

**Racemic:** When equal amounts of dextrorotatory and laevorotatory isomers are present, the resulting mixture has no optical activity, since the activities of each isomer cancels each other. Such a mixture is said to be **Racemic**.

**Resolution:** The separation of optically active isomers from a racemic mixture is called resolution.

### CYCLIC STRUCTURES

As the two reacting groups aldehyde and alcoholic group belong to the same molecule, a cyclic structure takes place. If the open-chain form of D-Glucose, which may be called as **Aldehydo-D-Glucose** is taken, and condense the aldehyde group on carbon-1, with the alcoholic-OH group on carbon-5, two different forms of glucose are formed.

**When the OH group extends to right, it is alpha-D-Glucose and it extends to left, it is beta-D-Glucose (Fig. 3.4).**

**Anomers and anomeric carbon:** Carbon-1, after cyclization has four different groups attached to it and thus it becomes now **asymmetric**. Compounds related in this way are called **anomers** and carbon-1, after cyclisation becomes asymmetric is called now **anomeric carbon atom (Fig. 3.4).**

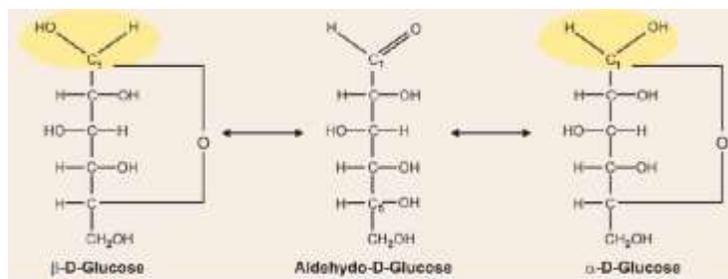


Fig. 3.4 C<sub>1</sub> after cyclisation becomes asymmetric—it is called "anomeric" carbon and α-D-glucose and β-D-glucose are "anomers"

### MUTAROTATION

**Definition:** When an aldohexose is first dissolved in water and the solution is put in optical path so that plane polarized light is passed, the initial optical rotation shown by the sugar gradually changes until a constant *fixed* rotation characteristic of the sugar is reached. This phenomenon of change of rotation is called as **mutarotation**.

### HAWORTH PROJECTION

(a) **Pyranoses:** Haworth in 1929 suggested that the six-membered ring forms of the sugars be called **Pyranoses**, because Pyran possesses the same ring of 5 carbons and oxygen.

(b) **Furanoses:** Similarly Haworth designated sugar containing 5-membered rings as the *furanoses*, because furan contains the same ring.

**Epimers and epimerisation:** Two sugars which differ from one another only in configuration around a single carbon atom are termed **Epimers**.

**Examples • Glucose and galactose are examples of an epimeric pairs which differ only with respect of C4 (Fig. 3.7).**

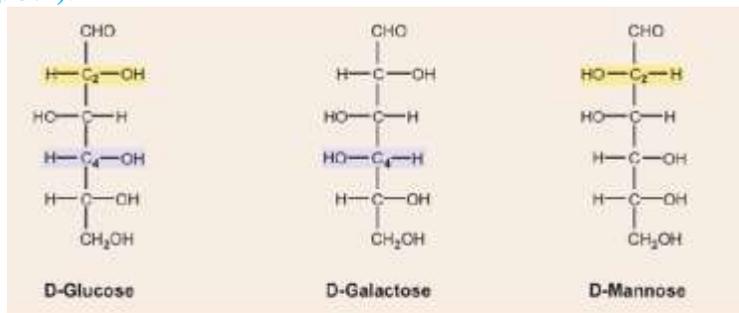


Fig. 3.7: Epimers

Similarly, mannose and glucose are epimers in respect of C2.

**Epimerisation:** Process by which one epimer is converted to other is called epimerisation and it requires the *enzyme epimerase*, e.g. conversion of galactose to glucose in liver.

## MONOSACCHARIDES

### MONOSACCHARIDES OF BIOLOGICAL IMPORTANCE

(a) **Trioses:** Both D-glyceraldehyde and dihydroxyacetone occur in the form of phosphate *esters*, as intermediates in glycolysis. *They are also the precursors of glycerol*, which the organism synthesises and incorporates into various types of lipids.

(b) **Tetroses:** Erythrose-4-P occurs as an intermediate in hexosemonophosphate shunt which is an alternative pathway for glucose oxidation.

#### (c) Pentoses

- D-ribose is a constituent of nucleic acid *RNA*; also as a constituent of certain coenzymes, e.g. FAD, NAD, coenzyme A.
- D-2-deoxyribose is a constituent of *DNA*.
- Phosphate esters of ketopentoses—D-ribulose and D-xylulose occur as intermediates in HMP shunt.
- L-xylulose is a metabolite of D-glucuronic acid and is excreted in urine of humans afflicted with a hereditary abnormality in metabolism called *pentosuria*.
- L-fucose (methyl pentose): occurs in glycoproteins.
- *D-Lyxose: It forms a constituent of lyxoflavin* isolated from human heart muscle whose function is not clear.

#### (d) Hexoses 1. D-Glucose: (Synonyms: Dextrose, Grape Sugar)

- It is the **chief physiological sugar** present in normal blood continually and at fairly constant level, i.e. about 0.1 per cent.
- All tissues utilise glucose for energy. *Erythrocytes and Brain cells utilise glucose solely for energy purposes.*
- Occurs as a constituent of disaccharide and polysaccharides.
- Stored as glycogen in liver and muscles mainly.
- Shows mutarotation.

**2. D-galactose:** Seldom found free in nature. In combination it occurs both in plants and animals.

*Occurs as a constituent of milk sugar lactose* and also in tissues as a constituent of galactolipid and glycoproteins.

- It is an **epimer of glucose** and differs in orientation of H and OH on carbon-4.
- It is less sweet than glucose and less soluble in water.
- It is dextrorotatory and shows mutarotation.

**3. D-fructose:** It is a ketohexose and commonly called as **fruit sugar**, as it occurs free in fruits.

• It is very sweet sugar, much sweeter than sucrose and more reactive than glucose. It occurs as a constituent of sucrose and also of the *polysaccharide inulin*. *It is laevorotatory and hence is also called laevulose*.

- Exhibits mutarotation.

### **Biomedical Importance**

**Seminal fluid is rich in fructose and sperms utilize fructose for energy.** Fructose is formed in the seminiferous tubular epithelial cells from glucose.

**4. D-mannose:** It does not occur free in nature but is widely distributed in combination as the polysaccharide mannan, e.g. in ivory nut. In the body, it is found as a constituent of glycoproteins.

**5. Sedoheptulose:** It is a ketoheptose found in plants of the sedum family. Its phosphate is important as an intermediate in the HMP-shunt and has been identified

### **Glycosides**

**Definition:** Glycosides are compounds *containing a carbohydrate and a noncarbohydrate residue in the same molecule*. In these compounds the carbohydrate residue is attached by an *acetal linkage* of carbon-I to the noncarbohydrate residue. **The noncarbohydrate residue present in the glycoside is called as Aglycone**. The aglycones present in glycosides vary in complexity from simple substances as methyl alcohol, glycerol, phenol or a base such as adenine to complex substances like sterols, hydroquinones and anthraquinones.

### **Biomedical Importance**

Glycosides are found in many drugs, spices and in the constituents of animal tissues. They are widely distributed in plant kingdom.

• **Cardiac glycosides:** It is important in medicine because of their action on heart and thus **used in cardiac insufficiency**. They all contain steroids as aglycone component in combination with sugar molecules. They are derivatives of digitalis, strophanthus and squill plants, e.g.

Digitonin  4 Galactose + Xylose + Digitogenin

### **DISACCHARIDES**

**Three** most common disaccharides of biological importance are: *Maltose, Lactose and Sucrose*. Their general molecular formula is  $C_{12}H_{22}O_{11}$  and they are hydrolysed by hot acids or corresponding enzymes as follows:



The disaccharides are formed by the union of two constituent monosaccharides with the elimination of one molecule of water. The points of linkage, the **glycosidic linkage** varies, as does the manner of linking *and the properties of the disaccharides depend to a great extent on the type of the linkage*. If both of the two potential aldehyde/or ketone groups are involved in the linkage the sugar will not exhibit reducing properties and will not be able to form osazones, e.g. *sucrose*. But if one of them is not bound in this way, it will permit reduction and osazone formation by the sugars, e.g. *Lactose and Maltose*.

## PROPERTIES OF DISACCHARIDES

**1. Maltose:** *Maltose or malt sugar* is an intermediary in acid hydrolysis of starch and can also be obtained by enzyme hydrolysis of starch. In the body, dietary starch digestion *by Amylase* in gut yields maltose, which requires a specific enzyme *maltase* to form glucose. It is a rather sweet sugar and is very soluble in water. *Since it has one aldehyde 'free' or potentially free (Fig. 3.15) it has reducing properties, and forms characteristic*

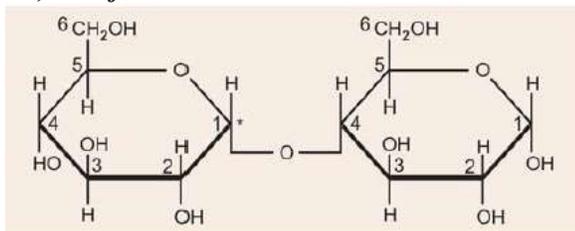


Fig. 3.15: Maltose ( $\alpha$  form)

*osazones*, which has characteristic appearance '*Sunflower*' like. As anomeric carbon of one glucose is free, can form  $\alpha$  and  $\beta$  forms and exhibit mutarotation. *On hydrolysis Maltose yields two molecules of glucose.*

**2. Lactose:** *Lactose is milk sugar* and found in appreciable quantities in milk to the extent of about 5 per cent and occurs at body temperature as an equilibrium mixture of the alpha and beta forms in 2:3 ratio. It is not very soluble and is not so sweet. It is *dextrorotatory*. Specific enzyme which hydrolyses is *lactase* present in intestinal juice. *On hydrolysis it yields one molecule of D-Glucose and one molecule of D-Galactose.* Because it contains galactose as one of its constituents, it yields *muic acid* on being treated with Conc HNO<sub>3</sub> after hydrolysis. *As one of the aldehyde group is free or potentially free (Fig. 3.16), it has reducing properties and can form osazones.*

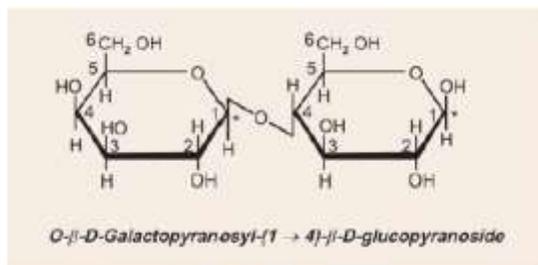


Fig. 3.16: Lactose ( $\beta$  form)

Lactosazone crystals have typical *hedgehog shape or powder puff appearance*. As anomeric carbon of glucose is **free**, can form  $\alpha$  and  $\beta$  forms and exhibits mutarotation.

**3. Sucrose:** *Ordinary table sugar is sucrose.* It is also called as 'Cane sugar', as it can be obtained from sugarcane. Also obtained from sugar beet, and sugar maple. Also occurs free in most fruits and vegetables, e.g. pineapples, and carrots. It is very soluble and very sweet and *on hydrolysis yields one molecule of D-Glucose and one molecule of D-Fructose.* The specific enzyme which hydrolyses sucrose is *sucrase* present in intestinal juice.

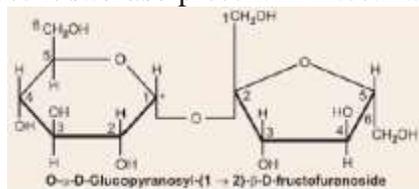


Fig. 3.17: Sucrose

*As both aldehyde and ketone groups are linked together, it does not have reducing properties, and cannot form osazones. As both anomeric carbons are involved **Invert Sugars and 'Inversion'** Sucrose is dextrorotatory (+62.5°) but its hydrolytic products are laevorotatory because fructose has a greater specific laevorotation than the dextrorotation of glucose. As the hydrolytic products invert the rotation, the resulting mixtures of glucose and fructose (hydrolytic products) is called as **Invert Sugar** and the process is called as **Inversion**. Honey is largely 'invert sugar' and the presence of fructose accounts for the greater sweetness of honey.*

### **Biomedical Importance of Disaccharides**

- Various food preparations, such as baby and invalid foods available, are produced by hydrolysis of grains and contain large amounts of maltose. From nutritional point of view they are thus easily digestible.
- In lactating mammary gland, the **lactose is synthesized from glucose by the duct epithelium** and lactose present in breast milk is a good source of energy for the newborn baby.
- Lactose is fermented by '**Coliform**' bacilli (**E. coli**) which is usually non-pathogenic (**lactose fermenter**) and not by **Typhoid bacillus** which is pathogenic (**lactose nonfermenter**). **This test is used to distinguish these two microorganisms.**
- '**Souring**' of milk: Many organisms that are found in milk, e.g. E. coli, Aerobacter aerogenes, and Str. lactis convert lactose of milk to lactic acid (LA) thus causing souring of milk.
- **Sucrose if introduced parenterally cannot be utilised**, but it can change the osmotic condition of the blood and causes a flow of water from the tissues into the blood. Thus **clinicians use it in oedema like cerebral oedema**. If sucrose or some other disaccharides are not hydrolysed in the gut, due to deficiency of the appropriate enzyme, diarrhoea is likely to occur.

### **OLIGOSACCHARIDES**

**Biomedical Importance:** Integral membrane proteins contain covalently attached carbohydrate units, oligosaccharides, on their extracellular face. Many secreted proteins, such as antibodies and coagulation factors also contain oligosaccharide units. These carbohydrates are attached to either the side-chain O<sup>2</sup> atom of serine or threonine residues by O-glycosidic linkages or to the side chain nitrogen of Asparagine residues by N-glycosidic linkages. N-linked oligosaccharides contain a **common pentasaccharide core** consisting of three mannose and two N-acetyl glycosamine residues. Additional sugars are attached to this common core in many different ways to form the great variety of oligosaccharide patterns found in glycoproteins.

*The diversity and complexity of the carbohydrate oligosaccharide units of glycoprotein suggest that they are rich in information and are functionally important. Carbohydrates participate in molecular targeting and cell-cell recognition.*

### **POLYSACCHARIDES**

- Polysaccharides are more complex substances. Some are polymers of a single monosaccharide and are termed as **Homopolysaccharides (Homoglycans)**, e.g. starch, glycogen, etc.
- Some contain other groups other than carbohydrates such as hexuronic acid and are called as **Heteropolysaccharides (heteroglycans)**, e.g. Mucopolysaccharides.

### **HOMOPOLYSACCHARIDES (HOMOGLYCANS)**

#### **1. Starch**

Starch is a **polymer of glucose**, and occurs in many plants as storage foods. It may be found in the leaves, and stem, as well as in roots, fruits and seeds where it is usually present in greater concentration.

- **Starch granules:** Appear under microscope as particles made up of concentric layers of material. They differ in shape, size and markings according to the source. Starchy foods are mainstay of our diet.

- **Composition of starch granule:** It consists of **two polymeric units** of glucose called (i) **Amylose** and (ii) **Amylopectin**, but they differ in molecular architecture and in certain properties (**Table 3.2**).

Amylose	Amylopectin
1. Occurs to the extent of 15 to 20%	1. Occurs 80 to 85%
2. Low molecular weight—approx. 60,000	2. High molecular weight—approx. 5,00,000
3. Soluble in water	3. Insoluble in water, can absorb water and swells up
4. Gives blue colour with dilute iodine solution	4. Gives reddish-violet colour with I <sub>2</sub> solution
5. <b>Structure</b> <ul style="list-style-type: none"> <li>• Unbranched</li> <li>• Straight chain</li> <li>• 250 to 300 D-Glucose units linked by α-1 → 4 linkages</li> <li>• Twists into a helix, with six glucose units per turn</li> </ul>	5. <ul style="list-style-type: none"> <li>• Highly branched structure</li> <li>• More D-Glucose</li> <li>• Units joined together</li> <li>• Structure similar to glycogen</li> <li>• Main stem has α-1 → 4 glycoside bonds</li> <li>• At branch point α-1 → 6 linkage, Approx 80 branches. One branch after every 24 to 30 D-Glucose units</li> </ul>

- **Solubility:** Starch granules are insoluble in cold water, but when their suspension is heated, water is taken up and swelling occurs, viscosity increases and **starch gels or pastes** are formed.
- **Reaction with I<sub>2</sub>:** Both the granules and the colloidal solutions react with Iodine to give a blue colour. This is chiefly due to amylose, which forms a deep-blue complex, which dissociates on heating. Amylopectin solutions are coloured blue-violet or purple.
- **Ester Formation:** Starches are capable of forming esters with either organic or inorganic acids.
- **Hydrolysis of starch:** It yields succession of polysaccharides of gradually diminishing molecular size.

**Enzyme (amylase) hydrolysis ends at maltose.** It is not quantitative conversion; traces of dextrans are also formed. For formation of glucose, it requires the enzyme **maltase**. **But if the hydrolysis is accomplished by acids much of the starch will be converted into glucose.**

- **Types of amylases:** Two broad classes of amylases exist as:

- **a-amylase** is present in **saliva** and **pancreatic juice**,

- **b-amylase** is present in sprouted grains and malts. Both of them hydrolyse only alpha-glycosidic linkage. alpha-amylase produces a random cleavage of glycosidic bonds well inside the starch molecule yielding a mixture of maltose and **some fragments larger than maltose (dextrans)**, whereas, **β-amylase splits off** maltose moieties liberating successive maltose units commencing at the non-reducing end of the starch molecule and **ends in**

**Limit dextrin.**

## 2. Glycogen

Glycogen is the reserve carbohydrate of the animal, hence it is called as **animal starch**. It has been shown to be present in plants which have no chlorophyll systems, e.g. in fungi and yeasts. It is also found in large amounts in oysters and other shell fish. **In higher animals, it is deposited in the liver and muscle as storage material which are readily available as immediate source of energy.** It is **dextrorotatory** with an  $[\alpha]_D^{20} = +196^\circ$  to  $+197^\circ$ . Formation of glycogen from glucose is called as **Glycogenesis** and breakdown of glycogen to form glucose is called as **glycogenolysis**. Postmortem glycogenolysis is very rapid but ceases when the pH falls to 5.5 due to lactic acid formed from glucose.

- **Molecular weight:** The molecular weight varies from 1,000,000 to 4,000,000.

- **Solubility:** Glycogen is not readily soluble in water and it forms an opalescent solution. It can be precipitated from opalescent solution by ethyl alcohol, and in drying, it forms a pure white powder.
- **Action of alkali:** Glycogen is not destroyed by a hot strong KOH or NaOH solution. *This property is made use of in the method for determining it quantitatively in tissues.*
- **Action with iodine:** Glycogen gives a deep-red colour. In this respect it resembles erythrodextrin.

**Structure:** Glycogens have a complex structure of highly branched chains. *It is a polymer of D-Glucose units and resemble amylopectin.* Glucose units in main stem are joined by a 1,  $\alpha$ 4 glucosidic linkages and branching occurs at branch points by a 1,  $\alpha$ 6 glucosidic linkage. A branch point occurs for every 12 to 18 glucose units

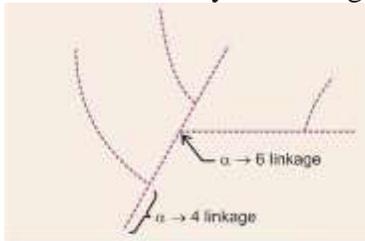


Fig. 3.19: Branched structure of glycogen—showing linkages

(Fig. 3.19).

### 3. Inulin

It is a **polymer of D-fructose** and has a low molecular weight (MW = 5000). It occurs in tubers of the Dehlia, in the roots of the Jerusalem artichoke, dandelion and in powder. It is *levorotatory* and gives no colour with iodine. Acids hydrolyse it to D-fructose; similarly it is also hydrolysed by the enzyme *inulinase*, which accompanies it in plants. *It has no dietary importance in human beings as inulinase is absent in human.*

#### Biomedical Importance

- It is **used** in physiological investigation for determination of the **rate of glomerular filtration rate (GFR)**.
- It has been also **used** for **estimation of body water (ECF)** volume.

### 4. Cellulose

Cellulose is a polymer of glucose. It is not hydrolyzed readily by dilute acids, but heating with fairly high concentrations of acids yields, the disaccharide *Cellulose* and D-Glucose. **Cellulose is made up of two molecules of D-Glucose linked together by beta-Glucosidic linkage between C1 and C4 of adjacent glucose units. Biomedical Importance**

Cellulose is a very stable insoluble compound. Since, it is the main constituent of the supporting tissues of plants, it forms a considerable part of our vegetable food. Herbivorous animals, with the help of bacteria, can utilise a considerable proportion of the cellulose ingested, **but in human beings no cellulose splitting enzyme is secreted by GI mucosa, hence it is not of any nutritional value.** But it is of considerable human dietetic value that it adds bulk to the intestinal contents (**roughage**) thereby **stimulating peristalsis and elimination of indigestible food residues.**

### 5. Dextrins

When starch is partially hydrolysed by the action of acids or enzymes, it is broken down into a number of products of lower molecular weight known as *dextrins* (see hydrolysis of starch). They resemble starch by being precipitable by alcohol, forming sticky, gummy masses.

#### Biomedical Importance

- Dextrin solutions are often **used as mucilages** (mucilages on the back of the postage stamp)
- Starch hydrolysates consisting largely of dextrans and maltose are widely **used in infant feeding**.

**Limit Dextrin:** It is a well-defined dextrin. This is the product remaining after the beta-*Amylase* has acted upon starch until no further action is observed.

## 6. Dextrans

It is a **polymer of D-Glucose**. It is synthesised by the action of *Leuconostoc mesenteroides*, a non-pathogenic gram +ve cocci in a sucrose medium. Exocellular enzyme produced by the organisms bring about polymerization of glucose moiety of sucrose molecule, and forms the polysaccharide known as **Dextrans**. They differ from dextrans in structure. They are made up of units of a number of D-Glucose molecules, having alpha1,6, alpha1, 4 or alpha1, 3 glycosidic linkages, within each unit and the units are joined together to form a network.

### Clinical Aspect

Dextran solution, having molecular wt approx. 75,000 have been **used as Plasma Expander**. When given IV, in cases of blood loss (haemorrhage), it increases the blood volume. Because of their high viscosity, low osmotic pressure, slow disintegration and utilisation, and slow elimination from the body they remain in blood for many hours to exert its effect.

**Disadvantage:** Only disadvantage is that *it can interfere with grouping and cross-matching*, as it forms false agglutination (Roleux formation). Hence **blood sample for grouping and cross-matching should be collected before administration of dextran in a case of haemorrhage and blood loss, where blood transfusion may be required**.

**7. Agar** It is a homopolysaccharide. Made up of repeated units of galactose which is sulphated. Present in seaweed. It is obtained from them.

### Biomedical Importance

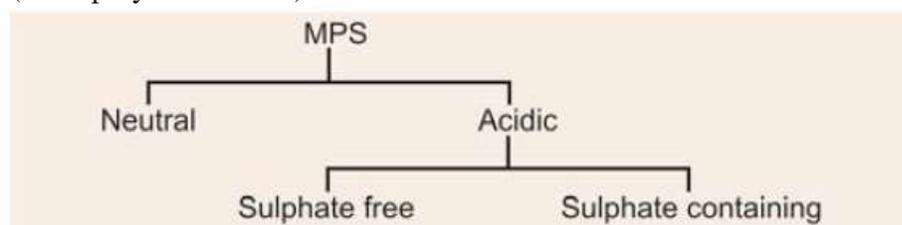
- **In human:** Used as **laxative** in constipation. Like cellulose, it is not digested, hence add bulk to the faeces (“roughage”value) and helps in its propulsion.
- **In microbiology:** Agar is available in purified form. It dissolves in hot water and on cooling it sets like gel. It is **used in agar plate for culture of bacteria**.

**HETEROPOLYSACCHARIDES (HETEROGLYCANS)— MUCOPOLYSACCHARIDES (MPS)** Jeanloz has suggested the name **Glycosaminoglycans (GAG)** to describe this group of substances. They are usually composed of **amino sugar** and **uronic acid** units as the principal components, though some are chiefly made up of amino sugar and monosaccharide units **without the presence of uronic acid**. The hexosamine present is generally acetylated. They are essential components of tissues, where they are generally present either in free form or in combination with proteins.

Carbohydrate content varies. **When carbohydrate content is > 4 per cent, they are called Mucoproteins and when < 4 per cent they are called as Glycoproteins.**

### CLASSIFICATION

Although there is no agreement on classification, the nitrogenous heteropolysaccharides (mucopolysaccharides) are classified as follows:



## I. Acidic Sulphate free MPS

### 1. Hyaluronic Acid

A sulphate free mucopolysaccharide. It was first isolated from vitreous humour of eye. Later it was found to be present in synovial fluid, skin, umbilical cord, haemolytic streptococci and in rheumatic nodule. It occurs both free and salt-like combination with *proteins and forms so called ground substance of mesenchyme*, an integral part of gel-like ground substance of connective and other tissues.

**Composition:** It is composed of repeating units of *N-acetyl glucosamine and D-Glucuronic acid*. On hydrolysis, it yields equimolecular quantities of D-Glucosamine, D-Glucuronic acid and acetic acid (Fig. 3.20).

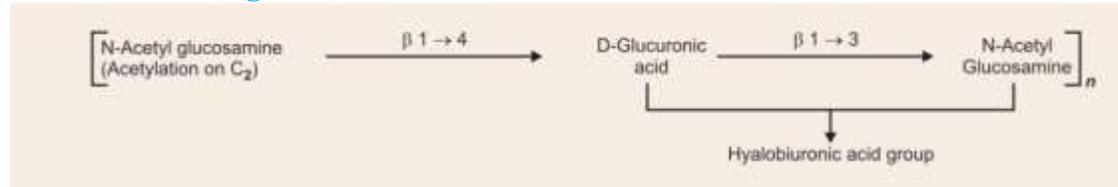


Fig. 3.20: Structure of hyaluronic acid

**Functions:**

- **Acts as lubricant in joints:** Hyaluronic acid in joints acts as a lubricant and shock absorbant. Intra articular injection of hyaluronic acid in knee joints is used to alleviate pain in chronic osteoarthritis of knee joints.

**Hyaluronidase:** An enzyme present in certain tissues, notably testicular tissue and spleen, as well as in several types of pneumococci and haemolytic streptococci. *The enzyme catalyses the depolymerisation of hyaluronic acid* and by reducing its viscosity facilitates diffusion of materials into tissue spaces. Hence the enzyme, sometimes, is designated as *spreading factor*.

#### Biomedical Importance

- The invasive power of some pathogenic organisms may be increased because they secrete **hyaluronidase**. In the testicular secretions, it may dissolve the viscid substances surrounding the ova to permit penetration of spermatozoa.
- **Clinically** the enzyme is used to increase the efficiency of absorption of solutions administered by clysis.

**2. Chondroitin** Another *sulphate free* acid mucopolysaccharide. Found in *cornea* and has been isolated from cranial cartilages. It differs from hyaluronic acid only in that it contains Nacetyl galactosamine instead of N-acetyl glucosamine.

## II. Sulphate Containing Acid MPS

**1. Keratan Sulphate (Kerato Sulphate)** A sulphate containing acid MPS. Found in costal cartilage, and cornea has been isolated from bovine cornea. It has been reported to be present in *Nucleus pulposus* and the wall of aorta.

**Composition:** It is composed of repeating disaccharide unit consisting of N-acetyl glucosamine and galactose. N-acetyl Galactose and N-Acetyl Glucosamine

*There are no uronic acids in the molecule.* Total sulphate content varies, but ester SO<sub>4</sub> is present at C6 of both N-acetyl glucosamine and galactose.

**Types:** **Two-types** have been described. They are found in tissues combined with proteins.

- **Keratan SO<sub>4</sub> I:** It occurs in cornea. In this type, linkage is between N-acetyl glucosamine and Asparagine residue to form the N-glycosidic bonding.
- **Keratan SO<sub>4</sub> II:** It occurs in skeletal tissues. In this type, the linkage to protein is by way of –OH groups on serine and threonine residues of the protein.

### 2. Chondroitin Sulphates

They are principal MPS in the ground substance of mammalian tissues and cartilage. They occur in combination with proteins and are called as **Chondroproteins**. **Four** chondroitin sulphates have been isolated so far. They are named as chondroitin SO<sub>4</sub> A, B, C and D.

**a. Chondroitin SO<sub>4</sub> A:** It is present chiefly in cartilages, adult bone and cornea.

**Structure:** It consists of repeating units of N-acetyl-D Galactosamine and D-Glucuronic acid. N-Acetyl galactosamine is esterified with SO<sub>4</sub> in **position 4** of galactosamine (Fig. 3.21).

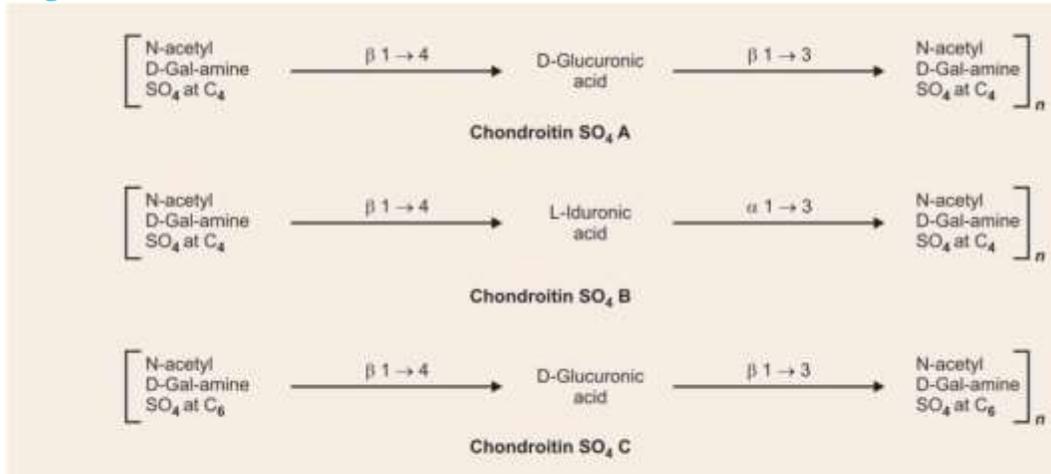


Fig. 3.21: Structure of chondroitin SO<sub>4</sub>

**b. Chondroitin SO<sub>4</sub> B:** It is present in skin, cardiac valves and tendons. Also isolated from aortic wall and lung parenchyma. **It has L-iduronic acid in place of glucuronic acid which is found in other chondroitin sulphates.** It has a **weak anticoagulant property**, hence sometimes it is called as □-**Heparin**. As it is found in *skin*, it is also called as **Dermatan sulphate**.

**Structure:** It consists of repeating units of **L-iduronic acid** and **N-acetyl galactosamine**. Sulphate moiety is present at C4 of N-acetyl galactosamine molecule.

**L-Iduronic acid:** It is an **epimer of D-Glucuronic acid**. Metabolically it is formed in the liver from D-Glucose.

**c. Chondroitin SO<sub>4</sub> C:** It is found in cartilage and tendons. Structure of chondroitin SO<sub>4</sub> C is the same as that of chondroitin SO<sub>4</sub> A except that the SO<sub>4</sub> group is **at position 6 of galactosamine** molecule instead of position 4.

**d. Chondroitin SO<sub>4</sub> D:** It has been isolated from the cartilage of shark. It resembles in structure to chondroitin

SO<sub>4</sub> C except that it has a second SO<sub>4</sub> attached probably at carbon 2 or 3 of uronic acid moiety.

### 3. Heparin

It is also called □-**Heparin**. **It is an anticoagulant present in liver** and it is produced mainly by **mast cells** of liver (Originally isolated from liver). In addition, it is also found in lungs, thymus, spleen, walls of large arteries, skin and in small quantities in blood.

**Structure:** It is a **polymer of repeating disaccharide units of D-Glucosamine (Glc N) and either of the two uronic acids-D-Glucuronic acid (Glc UA) and L-Iduronic acid (IDUA)** (Fig. 3.22).

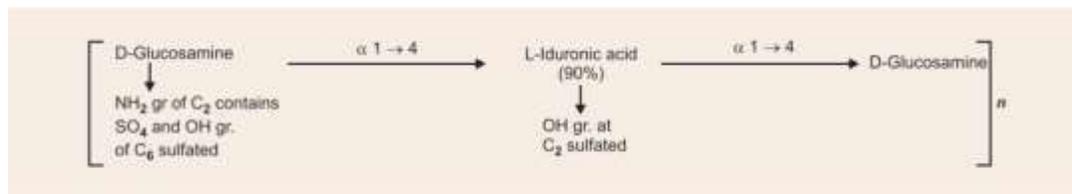


Fig. 3.22: Structure of heparin

The -NH<sub>2</sub> group at C<sub>2</sub> and OH group at C<sub>6</sub> of D-Glucosamine (Glc N) are sulphated. A few may contain acetyl group on C<sub>2</sub> of D-Glucosamine. In addition, the OH group of C<sub>2</sub> of uronic acids, D-Glucuronic acid and/or L-Iduronic acid, are sulphated. Initially, all of the uronic acids are D-Glucuronic acid (Glc UA), but “**5-epimerase**” enzyme converts approximately 90 percent of the D-Glucuronic acid residues to L-Iduronic acid (IDUA) after the polysaccharide chain is fully formed. **Hence, in fully formed Heparin molecule 90 per cent or more of uronic acid residues are L-Iduronic acid.**

**Properties:** It is **strongly acidic** due to sulphuric acid groups and readily forms salts. Molecular weight of Heparin varies from 17,000 to 20,000. It occurs in combination with proteins as proteoglycans. The protein molecule of heparin proteoglycan is unique, consisting chiefly **Serine** and **Glycine** residues. Approximately 2/3 of the serine residue contain GAG chains. Linkage with protein molecule is usually with GalN and serine/ sometimes with threonine.

**Functions:** (See below under Proteoglycans).

**Heparin antagonist:** The anticoagulant effects of heparin can be antagonised by strongly cationic polypeptides such as **protamines**, which bind strongly to heparin, thus inhibiting its binding to antithrombin III.

### CLINICAL ASPECT

**Inherited Deficiency:** Individuals with inherited deficiency of antithrombin III have been reported who are prone to develop frequent and widespread clots.

### 4. Heparitin Sulphate

Isolated from **amyloid liver**, certain normal tissues such as human and cattle aorta, and from the urine, liver and spleen of patients with gargoylism (Hurler’s syndrome).

**This compound has negligible anticoagulant activity.** It seems to be structurally similar to heparin, but has a:

- Lower molecular weight,
- Some of the amino groups carry acetyl groups and percentage of -SO<sub>4</sub> groups are smaller.
- Unlike heparin, its predominant uronic acid is D-Glucuronic acid (Glc UA).

Recently it has been shown that it is present on cell surfaces as proteoglycan and is extracellular.

### III. Neutral MPS

• Many of the neutral nitrogenous polysaccharides of various types are found in pneumococci capsule. Type specificity of pneumococci resides on specific polysaccharides present on capsule (“haptens”). Preparations of capsular polysaccharides from Type-1 pneumococci yield on hydrolysis glucosamine and Glucuronic acids.

• **Blood group substances:** These contain peptides or amino acids as well as carbohydrates. **Four monosaccharides** are found in all types of blood group substances regardless of source: **Galactose, fucose, Galactosamine (acetylated) and acetylated glucosamine.**

Non-reducing end groups of acetyl glucosamine, galactose and **fucose** are associated with blood group specificities of A, B and H respectively (**Fig. 3.23**).

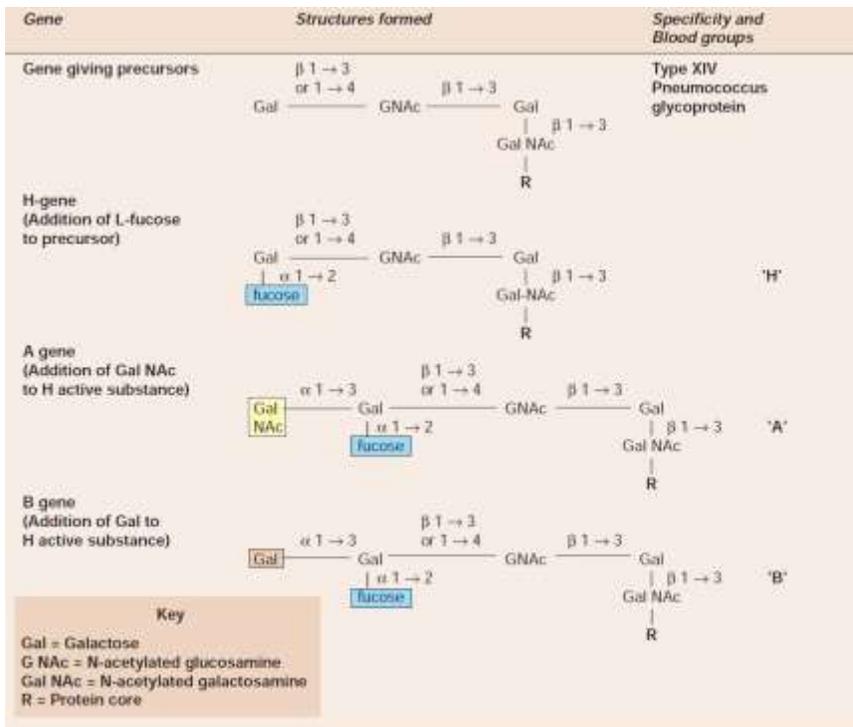


Fig. 3.23 Structure of blood groups—ABO system

The amino acid composition of blood group substances is peculiar in that S-containing and aromatic amino acids are absent.

- Nitrogenous neutral MPS firmly bound proteins, e.g. ovalbumin (contains mannose and glucosamine).

## PROTEOGLYCANS—CHEMISTRY AND FUNCTIONS

### Chemistry

- Proteoglycans are conjugated proteins. Proteins called “core” proteins are covalently linked to glycosaminoglycans (GAGs).
- Any of the GAGs viz. hyaluronic acid (HA); keratin sulphates I and II, chondroitin sulphates A, B, C, heparin and heparan sulphate can take part in its formation.
- The amount of carbohydrates in proteoglycans is much greater (upto 95%) as compared to glycoproteins.

**Linkages:** Three types of linkages between GAG and coreprotein is observed.

- **O-glycosidic linkage:** Formed between N-acetyl galactosamine (GalNAc) and serine or threonine of the core protein. **Example:** Typically seen in keratan SO<sub>4</sub> II.
- **N-glycosylamine linkage:** Formed between N-acetyl glucosamine (GlcNAc) and amide N of asparagine (ASn) of core protein.

**Example:** Typically seen in keratan SO<sub>4</sub>I and N-linked glycoproteins.

- **O-glycosidic linkage:** Formed between xylose (Xyl) and serine of the protein. This **bond is unique to proteoglycans.**

### Functions of Proteoglycans

- **As a constituent of extracellular matrix or ground substance:** Interacts with collagen and elastin
- **Acts as polyanions:** GAGS present in proteoglycans are polyanions and hence bind to polycations and cations such as Na and K. Thus attracts water by osmotic pressure into extracellular matrix contributing to its turgor.

- **Acts as a barrier in tissue:** Hyaluronic acid in tissues acts as a cementing substance and contributes to tissue barrier which permit metabolites to pass through but **resist penetration by bacteria and other infective agents.**
- **Acts as lubricant in joints:** Hyaluronic acid in joints acts as a lubricant and shock absorbant. Intraarticular injection of hyaluronic acid in knee joints is used to alleviate pain in chronic osteoarthritis of knee joints.
- **Role in release of hormone:** Proteoglycans like hyaluronic acid are present in storage or secretory granules, where they play part in release of the contents of the granules.
- **Role in cell migration in embryonic tissues:** Hyaluronic acid is present in high concentration in embryonic tissues and is considered to play an important role in cell migration during morphogenesis and wound repair.