CONTENTS

1.1 *Isolation, Purification and Characterisation*

1.2 *Structure and Kinetics*

1.3 *Immobilization*

1.4 *Effect of Toxic Chemicals*

CHAPTER 1

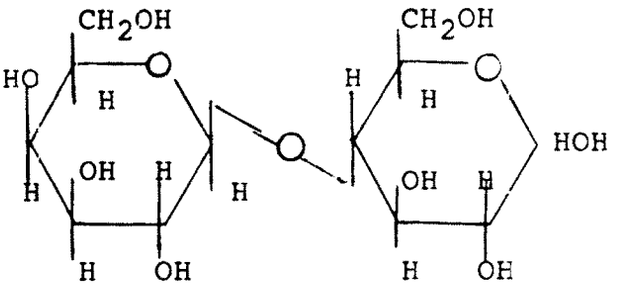
INTRODUCTION

Carbohydrates supply the major portion of the daily energy requirements of a normal individual; in an ordinary diet more than half of the total daily calories usually come from the carbohydrates. In addition to its use as a source of energy, carbohydrates are deposited as starch and glcogen, which constitute the major storage component of the plant and animal kingdom. It is metabolized to supply the carbon skeleton for certain amino acids, or may be converted into fat. Of these various processes, glycogen formation and breakdown occupies one of the central position in carbohydrate metabolism.

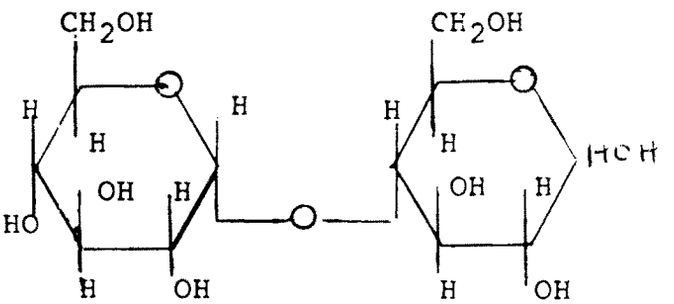
The principal carbohydrates in the food are :

(i) Polysaccharides :- Starch $(C_6H_{12}O_5)_n$ in vegetable foods; Cellulose and pectins.

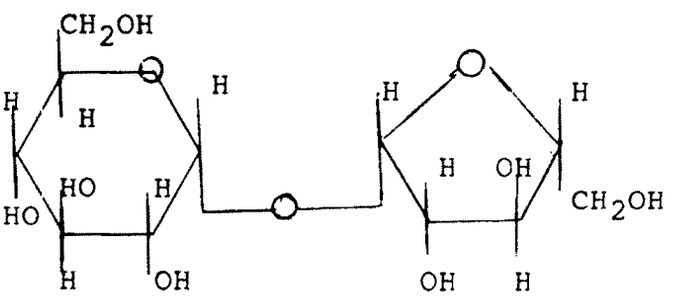
(ii) Disaccharides :- $(C_{12}H_{22}O_{11})$ for example; sucrose, beet - sugar, cane - sugar, lactose from milk and maltose.



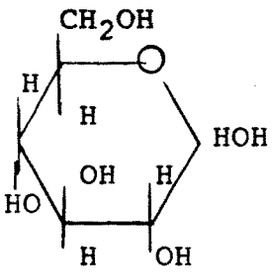
Lactose : galactose β- 1,4 glucose



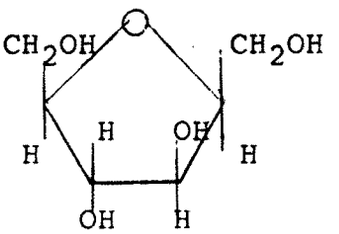
Maltose : glucose ∝- 1,4 glucose



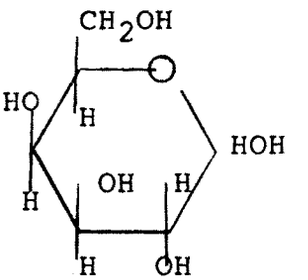
Sucrose : glucose ∝-1,2 fructose



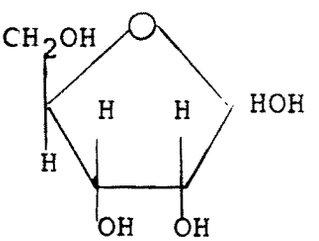
D - Glucose



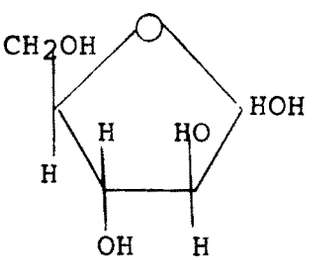
Fructose



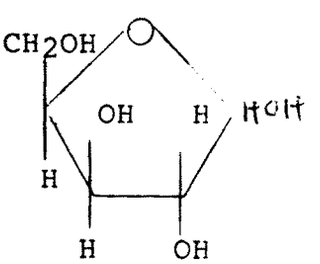
Galactose



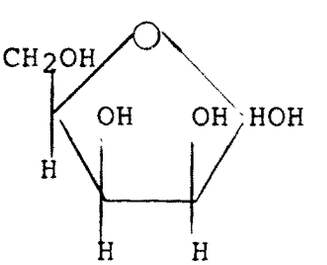
Ribose



Arabinose



Xylose



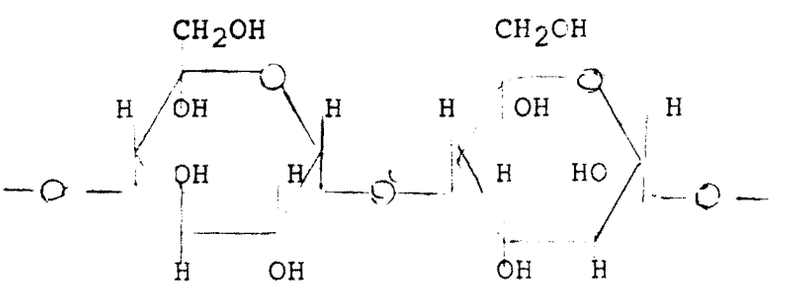
Lyxose

(iii) Monosaccharides :- (a) Hexoses ($C_6H_{12}O_6$) eg., glucose, fructose in fruits and vegetables.

(b)Pentoses (C_5H10O_5) They do not occur in the free form, but in nucleic acid and in certain polysaccharides eg., the pentosans of fruits and gums.

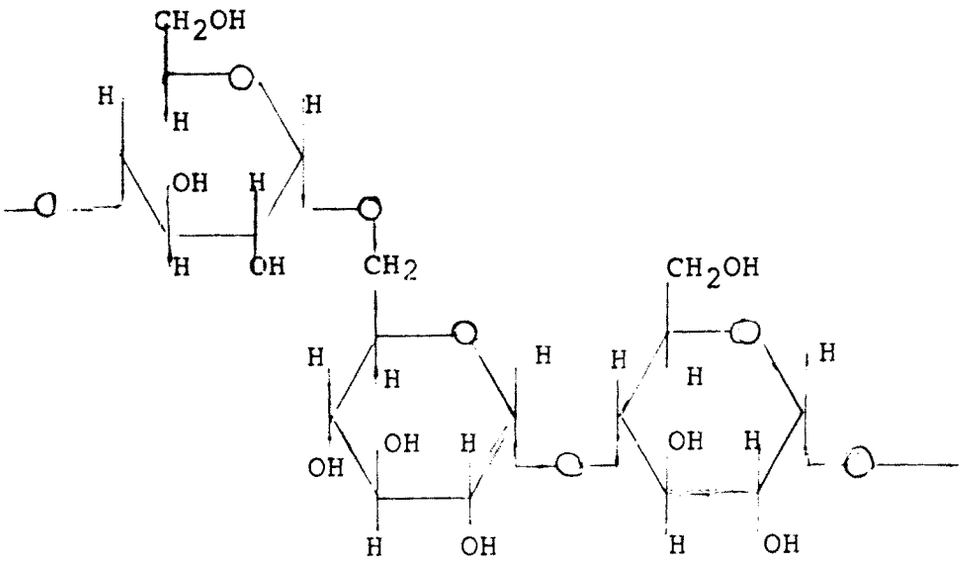
The polysaccharides are complex carbohydrates of high molecular weight. The constituent units of the polysaccharide molecule appear to be arranged in the form of a long chain which may be either branched (glycogen, amylopectin) or unbranched (cellulose, amylose). The linkage between the units is generally the 1,4 or 1,6 glycosidic bond, with either α- or β- configuration.

Starch is usually distributed throughout the vegetable kingdom. It occurs in grains, fruits, and tubes. It is found in the form of granules. The granules differ in size depending upon the source and they also differ to some extent in composition. The chief constituents are amylose and amylopectin. The linkage between the glucose units in unbranched amylose chain is the α- 1,4 glycosidic bond,



Amylo Chain Portion

In the branched chain structure of amylopectin, both α- 1,4 and 1,6 glycosidic bonds are found,



Dextrins are found as intermediate products in the course of hydrolysis of starch to glucose or maltose by acids or by enzymes.

Glycogen resembles starch and dextrins in the yield of the hydrolytic products, but differs significantly from these compounds in molecular architecture. In glycogen molecule the component glucose residues are linked in chains by both 1,4 and 1,6 α- glycosidic bonds similar to those in amylopectin. The branching in glycogen is much greater as compared to that found in starch or dextrins. This branching makes glycogen a more soluble compound than the other polysaccharides.

Glycogen is soluble in cold water to form an opalescent solution, and it gives a red colour with iodine. Some forms of glycogen however give blue or purple colour with iodine, these colour differences being related to the degree of chain branching (1).

Glycogen is found in varying amounts in all the tissues of the body, for example the glycogen content of the brain is very low as compared to that of muscle. Liver contains most of the glycogen store of the body. Glycogen is a suitable form to store carbohydrates as :

(i) It exerts negligible osmotic pressure and so does not disturb the intra cellular fluid content and does not diffuse from its storage sites.

(ii) It has a high energy level than the corresponding weight of glucose.

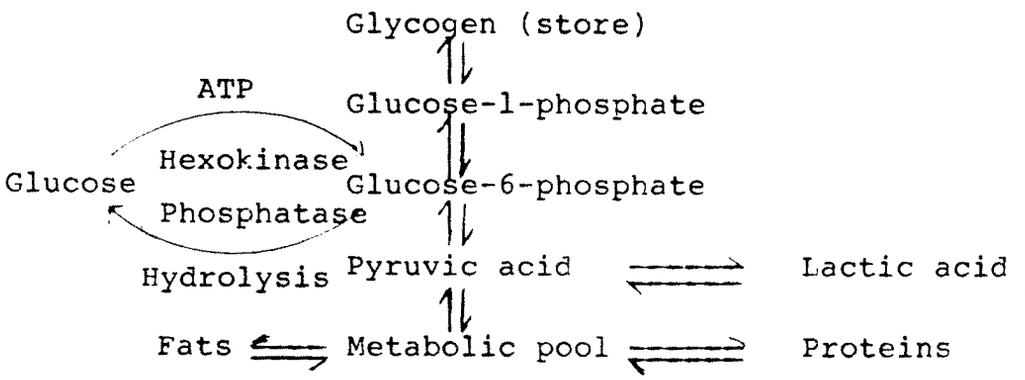
(iii) It can be degraded on demand for the benefit of other tissues, especially the brain and erythrocytes during fasting (2).

Muscle glycogen is formed from circulating blood glucose; the concentration in resting muscle is 0.7 to 1.0 percent. The rate of formation of muscle glycogen is increased by a rise in the blood glucose and by insulin

injection. It is decreased after the removal of pancreas, and can be restored to the normal level by injecting insulin. Muscle glycogen is consumed during muscular activity, when the muscle is rested after activity the glycogen store is built up again from blood glucose. Muscle glycogen cannot be readily converted back to blood glucose.

Glycogen is normally stored in the liver and is readily converted under ischaemic or anaerobic conditions into glucose. The average liver glycogen concentration is 5 gm percent. In the liver cells it is found in large granules composed from smaller granules in cluster; having highly branched glycogen molecules with a molecular weight of several millions.

Glycogen is formed in the liver and muscles via stages as indicated in the figure given below :



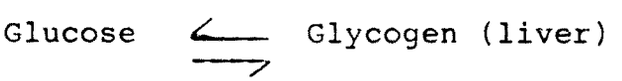
Blood Glucose & Liver Glycogen Metabolism

Liver glycogen is formed from

1) hexose monosaccharides end-products of carbohydrate digestion i.e. glucose, fructose, galactose, etc.;
2) intermediates of carbohydrate degradation especially, lactic acid and pyruvic acid,
3) glycerol obtained from hydrolysis of neutral fat and,
4) intermediates from degradation of amino acids (proteins) which enters in metabolic pool.

Liver glycogen functions in several ways. First and foremost, it is the only immediate available reserve of blood glucose. A high liver glycogen level protects the liver cells against the harmful effects of many poisons, e.g. carbon tetra chloride, ethyl alcohol, and various bacterial toxins. The degree of determination of amino acids in liver is depressed by rising levels of glycogen. It indicates that amino acids are preserved longer in that form and so remain available for protein synthesis in the tissues. Similarly, a high level of liver glycogen depresses the rate of ketone formation from long chain fatty acids and thus, abolishes or prevents ketosis.

The metabolism of brain is remarkable as adult mammalian brain normally uses only glucose as its food. The brain has very active respiratory metabolism. It uses almost 20 percent of total oxygen consumed by the resting human adults. Moreover, the use of oxygen by brain is fairly constant in rate, does not change significantly during the active thought or sleep. Because the brain contains very little glycogen, it depends on the incoming glucose from the blood, on minute to minute basis. If the blood glucose should fall significantly below certain critical levels for even short period of time, severe and sometimes irreparable changes in the brain function may occur. The liver in the intact animal assists to maintain the normal level of blood glucose in three main ways (3). Firstly, by regulation of reversible reaction,



Secondly, by regulating new glucose formation mainly from non-nitrogenous residues of amino acids produced from proteins, and the glycerol from fat degradation. Thirdly, by regulating the removal of glucose from blood and deposition as fat in liver.

Regulation of Reversible Reaction

Glucose $\rightleftharpoons$ Glycogen (liver)

Hexose sugars absorbed from intestine after carbohydrate meal increase the blood glucose level and lead to glycogen deposition. The liver is affected directly by the amount of blood sugar reaching it. A local rise of blood sugar increases glycogen deposition, on the other hand a fall of local blood sugar stimulates glycogen degradation and thus, sets up the secretion of glucose into the blood. Glycogenesis at constant blood sugar level is stimulated by harmones like insulin and adrenal corticoids.

Glycogen is converted into glucose whenever blood sugar tends to fall. The fall of blood sugar not only acts directly on liver but also on the central nervous system and indirectly cn the liver.

Liver glycogen is diminished as may be expected in muscular exercise, exposure to cold and starvation. The injection of insulin to normal individual produces hypoglycemia. The liver response in each case is compensatory reaction to hypoglycemia.

When blood glucose tends to rise the reaction (Glucogen $\rightarrow$ Glucose) ceases & reactions (Glucose $\rightarrow$ Glucogen) and (Glucose $\rightarrow$ Fatty Acid) commence and counteract by

removing the excess of glucose from the blood. When blood glucose level tends to fall, the reaction,

$$\text{All Glucogen} \Longrightarrow \text{Glucose}$$

proceeds. Thus, liver can be described as blood glucostat reacting to maintain the blood glucose within normal range.

The metabolism of liver glycogen is controlled glycogen synthetase and glucogen phosphorylase. These enzymes exist in two forms, ' a ' - active and ' b ' - inactive. The two forms are interconvertible through phosphorylation by Kinases and dephosphorylation by phosphatases (4). Glycogen synthesis occurs when D-glucose is phosphorylated to glucose-6-phosphate by hexokinase, the product is reversibly converted to glucose-1-phosphate by phospho-glucomutase enzyme. Glucose-1-phosphate uridyl transferase catalyzes UDP-Glucose (uridine-diphosphate glucose) formation from glucose-1-phosphate and UTP. UDP-Glucose acts as dcnar of glucose to form glycogen under the influence of glycogen synthetase by the reaction UDP-Glucose with ' primer ' (1, 4 polyglycon). The UDP-Glucose transfer it to non-reducing end of primer.

Dephosphorylated form of synthetase is active. When it is dephosphorylated by ATP at two serine hydroxyl groups by protein kinase, glucogen synthetase is converted to its

less active glycogen synthetase b. The conversion of the less active glycogen synthetase b back into active form is promoted by phosphoprotein phosphatase which removes the phosphate groups from serine residues.

Glycogen branching enzyme glycosyl 1, 6 transferase catalyzes the transfer of terminal oligosaccharide from non-reducing end of glycogen having at least eleven residue to a 6-hydroxy group of glucose residue of same or other glycogen molecule; thus, creating a branching in molecules (5).

The degradation of glycogen is catalyzed by phosphorylase enzymes. The phosphorylase a (an active form) on dephosphorylation by phosphorylase phosphatase yields phosphorylase b (an inactive form). The enzyme phosphorylase kinase converts phosphorylase b to phosphorylase a. Conversion of phosphorylase a to phosphorylase b involves hydrolysis of phosphate group at the sub-unit by phosphorylase a-phosphatase. The reverse conversion of it requires input of ATP and phosphorylase 6-kinase.

Hormone glucagan and epinephrine stimulates the synthesis of C-AMP level in the cell. It activates protein kinase, the protein-kinase inturn, activates phosphorylase

kinase. As a result of inactivation phosphorylase b is converted to phosphorylase a, which degrades glycogen to glucose-1-phosphate (6).

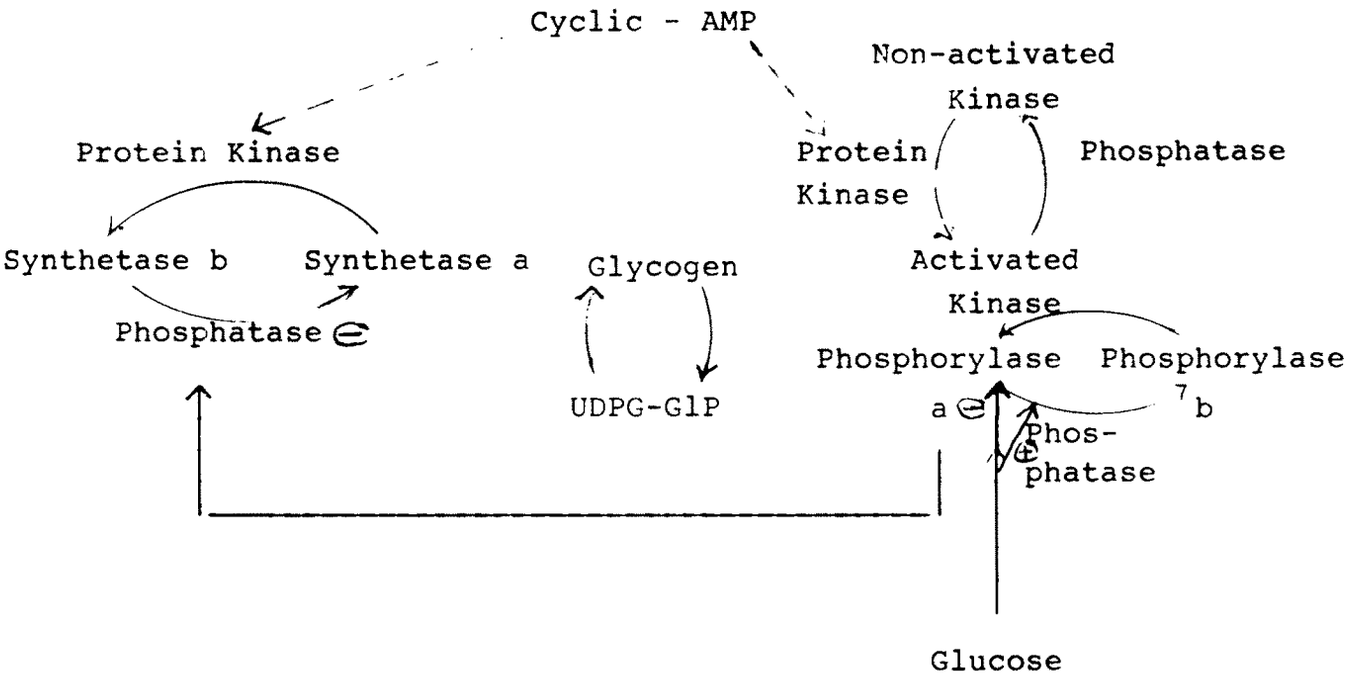
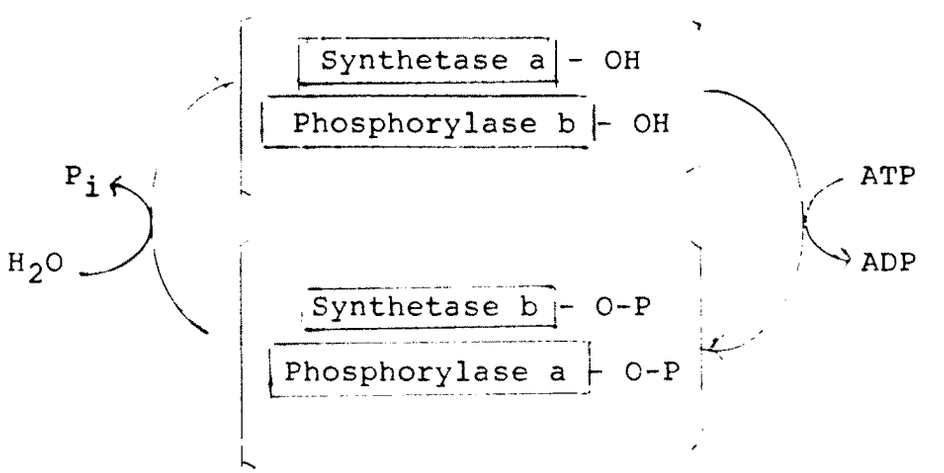


FIG. Control of Glycogen Metabolism in Liver (4)

The enzymes glycogen synthetase and glycogen phosphorylase are linked to their phosphorylation and dephosphorylation, and are regulated in a reciprocal manner. While one enzyme is activated, the other is inhibited (5).



Glycogen storage diseases or glycogenoses are group of genetically determined conditions that are characterised by the deficiency of one of the enzymes concerned with the degradation or synthesis of cellular glycogen. The effect may result in the presence of abnormal concentrations of glycogen in one or more organs or tissues and also the accumulation of glycogen having unusual molecular structure. In 1952, Gerty and Cori (7) reported glycogenoses due to the deficiency of hepatic glucose-6-phosphatase. Four years later the same group of scientists demonstrated that another type of glycogenosis results from a lack of amylo-1,6-glucosidase.

Human glycogenoses type III arises from the deficiency of amylo-1,6-glycosidase with major clinical features such as, hepatomegaly with variable cardiomegaly and hypotonia. Normal glycogen response in the fed state is poor to good prognasia.

The Disease Type IV arises due to the deficiency of amylo-1,4 - 1,6 trans glycosylase with symptoms such as hepato-spleeno-megaly, portal cirrhosis, ascites, death usually in early childhood.

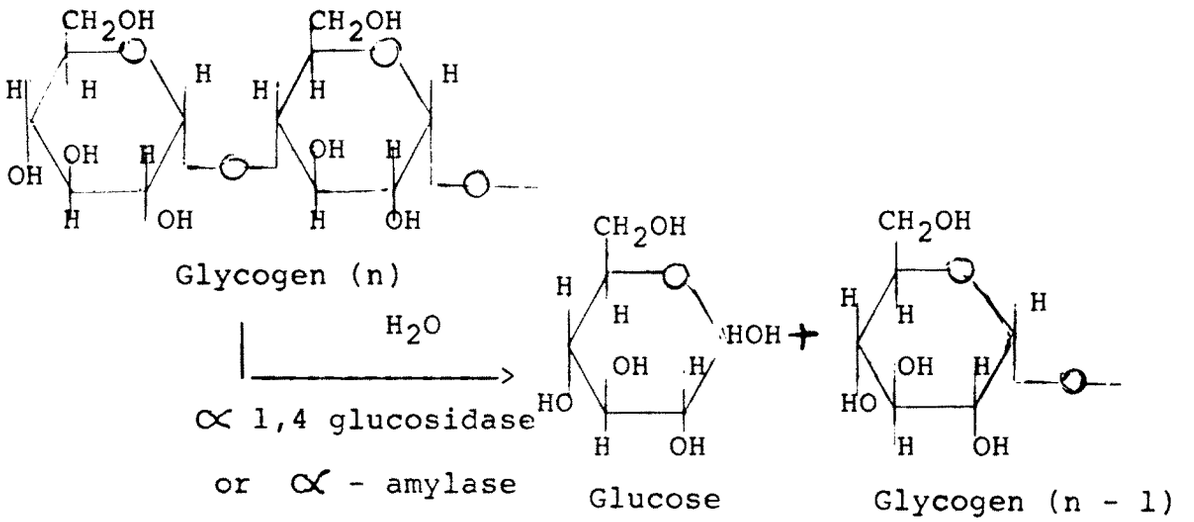
In both the types of diseases liver, muscle, and heart tissues are affected in different combinations. The occurance of both these diseases is very rare.

The liver glycogen content depends upon the physiological condition of the animal, and varies considerably due to starvation and depending on the nature of diet. The variation could be from traces to as high as 8 percent of the weight of the liver tissue.

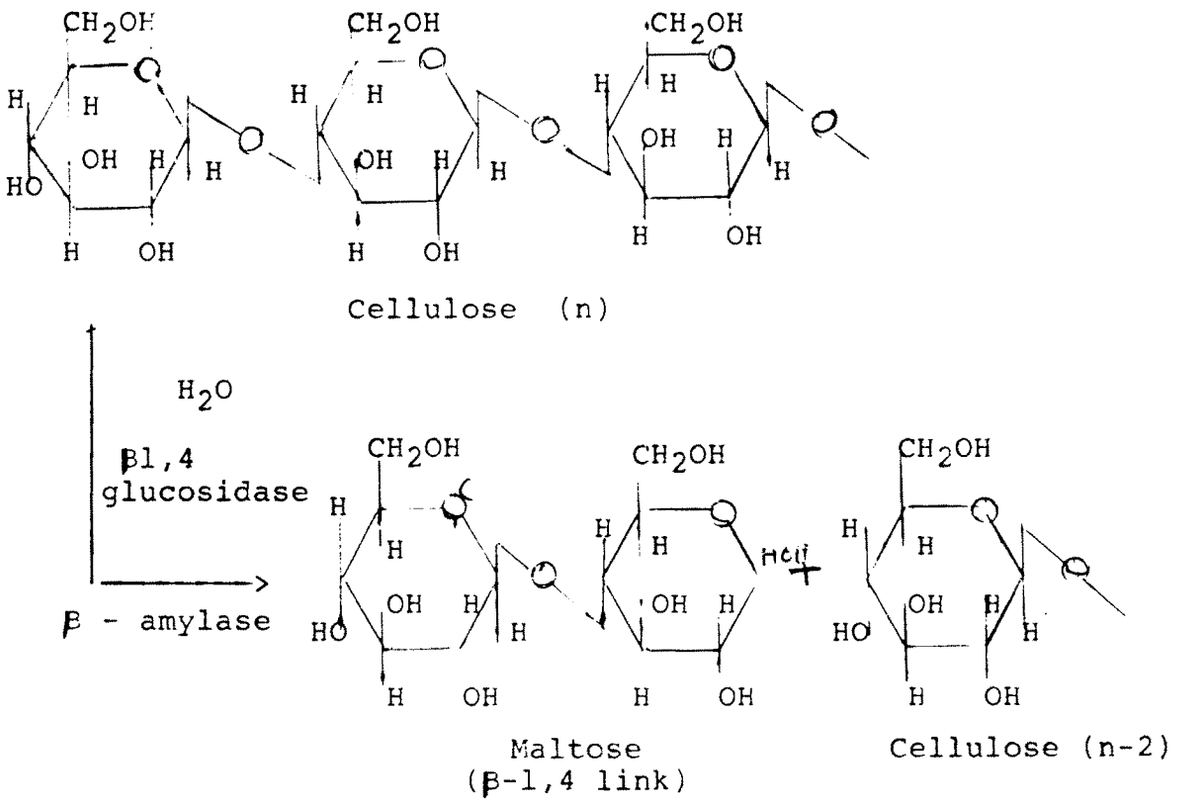
Glycogen is degraded to a limited extent by the enzyme phosphorylase alone. However, the 1,6 glycosidic bonds at the branch points are not susceptible to cleavage by phosphorylase. Phosphorylase is known to stop cleaving α -1,4 linkages when it is four residues away from the branch point. Another enzyme, probably glucoamylase is required at this stage (8). The transfer of three to four glycosyl residues takes place from one outer branch to adjacent non-reducing end. Further degradation occurs by the cleavage of the α -1,4 and 1,6 glycosidic linkages (9). Thus amylolytic enzymes are of great interest.

Amylases are classified into different groups according to their mode of hydrolysis of the substrate (10).

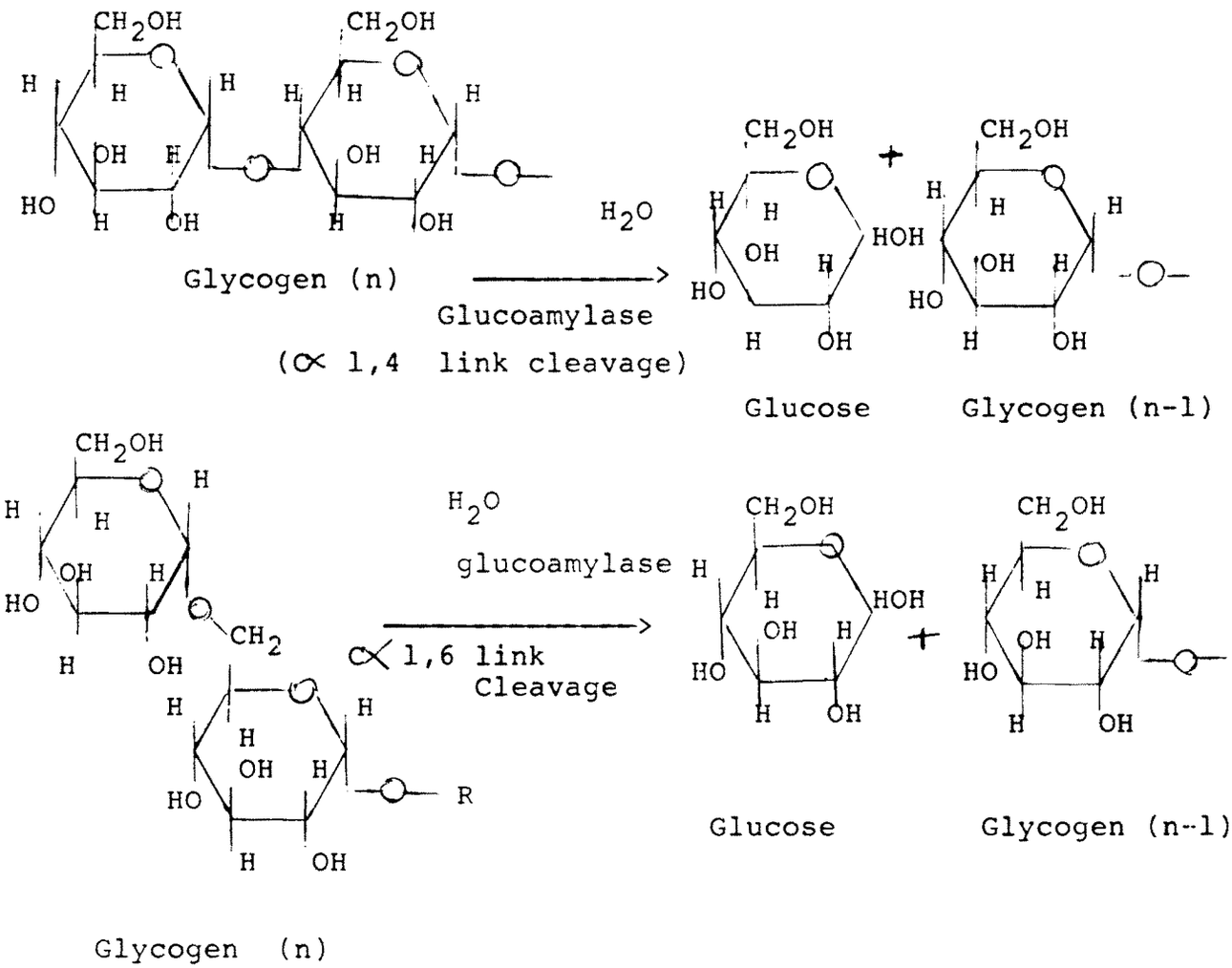
E. C. 3.2.1.1, ∝- amylase which hydrolyses ∝- 1,4 glucan link in polysaccharides. It hydrolyses in a random manner the ∝- 1,4 links in outer and inner chains beyond the branch point. Thus it causes rapid depolymerisation of gycogen to oligosaccharides (9).



E. C. 3.2.1.2., β - amylase, which hydrolyses β -1,4 linkage in polysaccharides so as to remove successive maltose units from non-reducing end of the chain.



The nomenclature of E. C. 3.2.1.3. is designated as glucoamylase, amyloglucosidase, γ - amylase. And it is designated according to their source or origin. Now a days E.C. 3.2.1.3. is synonymously called by workers as glucoamylase. Glucoamylase hydrolyses α - 1,4 linkage in polysaccharides so as to remove successive glucose units from the non-reducing end of the chain. The α- 1,6 glucan linkage is also hydrolysed by glucoamylase (11).



Liver glycogen plays an important role in maintaining the normal level of blood glucose. The amylases are mainly involved in degrading glycogen. The effect of substances which can either activate or inhibit the amylases is of prime interest, as they may directly affect the level of blood glucose.

Brain requires more glucose as compared to the other tissues. Hence it follows, that when amylases are either activated or inhibited the brain glucose supply via the blood glucose is altered, and it may manifest in clinical changes in the functioning of the brain. This aspect is overlooked in the treatment of patients with drugs that may cause the activation or inhibition of the enzyme and thus precipitate serious clinical conditions.

An attempt has been made to study the effect of certain drugs on the activity of liver glucoamylase. Further effects of drugs on free and immobilized enzyme has been highlighted.

1.1 ISOLATION, PURIFICATION AND CHARACTERIZATION

Glucoamylase (12) / Amyloglucosidase (15) / α-1,4 - amylase (14) / α-1,6 glucan hydrolase (13), E. C. 3.2.1.3. is commonly referred to as glucoamylase in the present investigation is widely distributed in nature. It primarily catalyzes the degradation of α- glucans (starch, glycogen, dextrins) and is very specific towards the hydrolysis of α -1,4 linkages from the non-reducing end. This exoenzyme also carries out the hydrolysis of α- 1,6 glucan linkages, but at a slower rate. The enzyme activity appears to be reversible under certain set of conditions (13).

In 1987 DE-Mote Rene et al (16) purified and characterized the glucoamylase of extracellular origin from Candida antartica (CBS, 6678). The conventional procedure of enzyme purification was adopted to achieve an enzyme showing manomeric glycoprotein with a molecular weight 48,500 daltons, and pH, temperature optima 4.2, and 51°C respectively. The enzyme was strongly adsorbed onto raw starch granules. The major amylolytic enzyme in the culture fitrates of Candida cerebella grown in a media containing starch has been purified and charecterized as glucoamylase by N. J. King (12). The enzyme has a pH optima of 4.5.

Hg^{++} ions inhibited the activity at 5mM concentration. The action of the enzyme was studied using amylopectin, amylose and maltose. The enzyme hydrolysed both α-1,6 linkage at branching point and α- 1,4 linkage; glucose was the final product.

Hayun H. H. and Co-workers (17) carried out the biochemical characterization of the enzyme obtained from the cell extracts of Clostridium thermohydrosulfuricum. An aerobic fermentation by cell extracts at 65°C resulted in the formation of ethanol using starch as a substrate. The pH stability ranges from 4 - 5.6. The apparent Vmax using starch was 0.41 mg/ml and 0.31 units per mg of protein. The enzyme was active and stable in presence of air and 10 percent ethanol. The enzyme activity from the organism actively degraded starch even in the abscence of α- amylase.

The stability of the enzyme obtained from A. shirousmaii was found to be below 55°C, retained almost full activity after one hour, but it was labile over 70°C (18). At this temperature it lost almost all the activity within 10 minutes. The enzyme was most stable at pH 4.0. Similarly enzyme activity was inhibited by Hg^{++} ions. Ca^{++} ions markedly increased the heat stability of the enzyme

though it did not affect the kinetics of enzyme activity.

The ability of toxin producing strain A. clavatos (H.W.U.-G-DT) from malting barley to hydrolyze starch at 3°C was demonstrated (19). Maximum glucoamylase production was recorded on 4th day, a correlation was observed between mycelial growth and enzyme production. The enzyme was found most active at pH 5.2 and at 40°C.

Aspergilus niger releases intracellular glucoamylose (20), the process involves suspension of mycelia at stationary phase in water and homogenization and agitation of suspension. The released intra-cellular enzyme was purified by DEAE cellulose.

Glucoamylase from rat liver was isolated and characterised by Marjere Stetton (21). Rosenfield (14) isolated α -amylose free glucoamylase from rat liver; the enzyme showed maximum activity at pH 4.5 and had a molecular weight 90,000 daltons. The enzymes of similar activity was isolated and purified from rat liver by Peter Sefrey and D. H. Brown, Barbara I Brown (22), displaying the maximum activity at pH 3.7 for maltose, pH 4.2 for isomaltose, pH 4.4 for glycogen.

The glucoamylase from cattle liver displaying maximum activity at pH 4.5 and molecular weight of 107,000 daltons. The liver enzyme is called 4-glucosidase (23).

The muscle amylo 1-6 glycosidase, debranching enzyme, was identified by R. A. Dedonder (24). Enzyme purified from Endomyceus breaks α -1,4 as well as α -1,6 linkages and hydrolyzes glycogen and amylopectin completely. The enzyme also splits 1,3 linkages.

1.2 STRUCTURE & KINETICS

The kinetic studies and careful electrophoretic analysis using polyacryl amide gel electrophoresis (PAGE) and S D S - PAGE favoured isoenzyme patterns in Rhizopus niveus and A. niger (25).

The gluco amylases isolated from various sources are glycoproteins (26). The protein carbohydrate linkage, involving mannose o-glycosidically linked to serine and threonine. The majority of neutral oligosaccharides are located in a region of $\sim$ 70 amino acid residues which carries $\sim$ 35 oligosaccharides units (27).

Aspergilus awanmori glucoamylase has molecular weight 102,000 daltons, the carbohydrate moiety makes up for 7 percent of the total protein mass. Glycoprotein has a short oligomeric chains with mannose as the basic monomeric link (28).

A greater amino acid sequences of three glucoamylases from Rhizopus, Aspergilus, Saccharomyceus were compared by Tomoko Yashi Kazu (29), and overall homology was found from 25 to 36 percent. A greater homology was observed in four distinct regions favouring functionally active and similar amino acid sequences. Rhizopus and Aspergilus glucoamylases were more closely related amongst the enzymes studied. The structure and function relationship of glucoamylases is probably on secondary structure. According to Puzur (26) array of carbohydrate chains in glycoproteins may account for some unique properties.

Clarke Anthony (30) identified an essential tryptophan residue in primary structure of glucoamylase. The presence of carboxyl group in active site of enzyme is shown by Savelev A. N. (31).

Sankaran and others (32) studied the size and shape of rabbit intestinal glucoamylase - maltose complex. The

complex is an asymmetrical aggregate with molecular weight 250,000 - 760,000 daltons. It resembles a long string (6.2 nm) consisting of 8 beads of diameter 6.0 nm each and a surface to surface interbead distance of 20 nm. Beads height represents ~ 6 sub-units.

1.3 IMMOBILIZATION

The primary function of glucoamylase is to release glucose on demand. Little is known about the regulation of enzyme activity and about the response of this enzyme towards toxic environment. The enzyme protein must be flexible and must undergo conformational changes in order to align itself with the substrate affecting degradation of glycogen molecule. It is therefore, of great interest to study kinetics of free and immobilized glucoamylase in the presence of toxic environment.

Enzymes are very difficult to reuse, from reactions containing catalytic amount. Recovery of these catalyst is achieved by the process of immobilization. Immobilization is a process of limiting the movement of enzymes. The techniques of immobilizations involve,

1) adsorption of enzyme on a carrier surface,
2) Covalent coupling,

3) crosslinking between enzyme molecules,

4) entrapment of an enzyme in a matrix, or

5) confinement of an enzyme solution in a membrane structure known as encapsulation.

Each of immobilization process has both advantages and disadvantages. The choice is made depending upon the stability of immobilized enzyme and the nature of reaction system. The greater return from immobilization is achieved with expensive enzyme because such immobilized enzymes are active and stable for long periods of time.

Glucoamylase is easily available enzyme. Its immobilization would need to exhibit very superior properties as compared to the soluble enzyme. This is very true for commercial purposes.

The immobilization of glucoamylase using entrapment techniques were carried out by using various entrapers like poly acrylonitirle (33), 2-hydroxy methyl acrylate (34), polyvinyl alcohol (35), and cellulose acetate (36). Glucoamylase was microencapsulated in liquid membrane by Gregoridis and others (37).

Ionic binding and metal binding methods were used in glucoamylase immobilization include the ion-exchange resins and metal binders with inorganic supports (38,39).

Adsorption and covalent techniques in glucoamylase immobilization provided the information that highest stability was observed in covalent bonding. The binding efficiency and immobilization of enzyme preparation depend upon the nature of insoluble carriers and purity of enzyme. The choice of cross linking agent promoting a binding between enzyme and carriers is very significant (40).

SP. Sephadex bound enzyme exihibited lower stability than the free enzyme and greater stability than glucoamylase in presence of dextran sulphate (41). The thermal stability was improved by immobilization with bovine (35) albumin, chemical modification of glucoamylase affected activity and thermal stability of free and immobilized enzyme (42). Complete chemical modification caused significant loss in the activity and thermal stability.

Binding reactions and carriers are used in immobilization. These contain the functional group which on activation can be covalently coupled. This procedure provides simple and easy technique to immobilization, hence these are generally used.

In the present experiment, the non-biodegradable, stable agaropectin was activated with CNBr and linked to glucoamylase. The immobilized glucoamylase is water soluble could be separated on centrifugation and is quite stable at room temperature. The agaropectin glucoamylase complex has been used for converting starch into glucose - the technology is of commercial importance.

1.4 EFFECT OF TOXIC CHEMICALS

The glucoamylase activity is affected by toxic environement. The present investigation enlightens the effect of antibiotics on its activity. The matter is of great concern because the infections are heavily intoxicated with antibiotics. The release of glucose with such conditions is of vital importance.

The enzyme is a glycoprotein containing mannose, glucose, galactose and uronic acid, and has a molecular weight 90,000 to 100,000 daltons and pH optima of 4.3 to 4.5. The Ca^{++} ions increased the heat stability (18).

A reversible protective effect by EDTA in irradiation inactivation of enzyme was observed and had a maximum at certain EDTA concentration (43).

Size of starch granule has an effect on activity of the enzyme. Small granules had higher heat of gelatinization than those of large granules. Small granules of a non-waxy culture were degraded four times or more rapidly than large granules (44).

Peritonial injection of glucoamylase 100 unit/kg weight to female rabbit showed (46) marked increase in blood glucose and decrease in plasma inorganic phosphate. The maximum increase and decrease of inorganic phosphate accounted between 2nd - 3rd hour after injection and had not returned to the pre-injection values after five hours. Two hours after injection liver glycogen content was significantly lower than that of controls.

The effect of acid glucoamylase from animal tissue such as pig spleen, human liver, rabbit kidney on C -gluco-oligo saccharides containing (1 -> 2, 1 -> 3, 1 -> 4, 1 -> 6) glycosidic bonds was studied by Minakova and others (46). Tri-saccharides containing bonds of all types and tetra saccharide containing 1 ->3, 1 -> 4, 1 -> 6 glycosidic bonds are completely hydrolysed to glucose.

Cold exposure of rat for three hours (6 - 2°C) caused increase in amylase activity of sub-maxillary gland(47).

Administration of Actinomycin-D to the cold exposed rats produced a tremendous increase in enzyme activity instead of abolition of increase.

Fluorides selectively inhibited the cleavage of maltose, but no effect has been observed on cleavage of glycogen (48).

Concentration of dimethyl sulphoxide in water below 2.5 M do not affect the activity of enzyme, but progressive inhibition was observed at higher concentrations (44).

Purified glucoamylase enzyme was inactivated when dilute solutions (2 - 6 µg/ml) were kept in thin layer. On the other hand, crude preparations were not inactivated (50), and temperature upto 30°C increased the inactivation. Inactivation occured equally under H_2, O_2, or air. Further, the addition of milk casein, ovalbumin upto 100 Ug/ml tended to prevent inactivation.

The intrinsic rate constant K_m electrostatically to the dielectric constant of hydro-arganic solution (51). The affinity of 3rd subsite which affect apparent rate constant K_o was correlated with $\triangle$ G of maltose, amino acid side chains.

Factors which affect the activity includes Fe^{+++}, Co^{++}, Ca^{++}, Mg^{++} and had an activating effect (20%). Whereas, heavy metal ions Hg^{++}, Pb^{++}, Cu^{++} had inhibitory effect (80 percent) (52). Denaturation by sodium dodecyle sulphate, urea, and guanidine hydrochloride was reversible. Glucano-6-lactone and maltitol are competitive inhibitors. Some anions $A_5O_2^-$, $B_4O_7^{--}$, $SO3^{--}$ inhibited remarkably upto 94%, 95%, and 88% respectively (18). Erythritol also inhibits the activity (53).

Glucoamylase activity is inhibited by aminoalcohol derivatives (54). Hydrolysis of maltose was inhibited competitively by amino alcohol at pH 7 and Tris (hydroxy methyl) amino ethane, amino-2-ethylol-3-propan-diol and 2-2' amino cyclohexanol were relatively good inhibitors.

Nojirimycin and 1-hydroxy-nojirimycin derivatives are potent inhibitors of enzyme (55, 56) in the range of 1 - 5 µg/ml and are of competitive type.

1.5 APPLICATIONS OF GLUCOAMYLASE

Manufacture of dextrose by enzymic conversion of starch overcomes the drawbacks of acid hydrolysis method. The conversion takes place at pH 4 - 4.5 and at 140 - 145°F (57). Glucoamylase is used batchwise on large

scale in starch processing industry. The glucoamylase is used in dough preparation (58) at level of 1.5 units per gm flour.

Glucoamylase immobilized to acid washed charcoal was used to effect the saccharification of liquified cossova starch (59). A 7 DE (dextrose equivalent) liquified cossova starch (30% DS) was converted to 69 DE cossova syrup by glucoamylase, also removes brown colour of syrup.