CONTENTS

Chapter 1

GLYCOGEN

Introduction

THE polysaccharide glycogen is the major energy and carbohydrate reserve of the human body, and is stored widely in the liver, muscles, heart and other tissues. In many ways glycogen serves the same function in animals as starch does in plants, although it is found also in many microorganisms. Unlike starch which consists of two components, amylose (the linear polymer of α-1:4-D-glucopyranose units) and amylopectin (the branched polymer in which chains of α-1:4-D-glucopyranose units are joined by α-1:6-linkages), glycogen is generally accepted to be constituted of a single molecular species although it is highly polymolecular. Most glycogens have a highly branched structure comprising several hundred unit chains of *ca.* twelve α-1:4-linked D-glucopyranose residues with inter-chain links of the α-1:6-type. It thus resembles amylopectin to some extent, the major difference being that in the latter the average chain length is about twenty glucose residues. From the evidence presented below the structure of glycogen and its molecular size can be seen to vary somewhat from one animal to another and even in the various tissues of the same animal. Despite this, however, the overall structural details of the various glycogens are quite similar except during the onset of certain diseases. Since both the molecular size and structure of glycogen may be to some extent governed by the method of isolation employed this will be dealt with first.

Methods of Isolation

The two major methods of isolation of glycogen from tissues involve the use of either 5%–10% aqueous trichloracetic acid in the cold (0°) or the more conventional Pflüger (1905) extraction with 30% potassium hydroxide at 100°. In each method this extraction is followed by precipitation of the glycogen from solution by addition of ethanol. The products obtained by these methods and the effects of variation of these conditions have been the subject of extensive studies by Stetten, Katzen and Stetten (1956, 1958). These workers showed that the weight-average molecular weight of glycogen declined when dissolved in hot aqueous potassium hydroxide and that this decline was more rapid in 1N-KOH than in 10N-KOH and faster under oxygen than under an atmosphere of nitrogen. Less extensive degradation occurred using the trichloracetic acid method although even with this

reagent some degradation does occur. Thus the molecular weight of a sample of liver glycogen dissolved in 5% aqueous trichloracetic acid fell from 71×10^6 to 44×10^6 at 0° in 2 hours and to 14×10^6 at 23° in the same time. Using the trichloracetic acid method glycogens were obtained from rabbit liver, rabbit muscle, and rat leg muscle having molecular weights of $45{\cdot}2 \times 10^6$, $11{\cdot}9 \times 10^6$ and $43{\cdot}8 \times 10^6$ respectively. From the same sources values of $2{\cdot}7 \times 10^6$, $3{\cdot}1 \times 10^6$ and $6{\cdot}1 \times 10^6$ were obtained for glycogens isolated by the potassium hydroxide method. These results are not in agreement with those obtained much earlier by Staudinger (1942) and Bridgman (1942) who reported that glycogen extracted with cold trichloracetic acid and hot alkali from two halves of a rabbit liver had a similar molecular weight.

Bryce, Greenwood, Jones and Manners (1958) have studied the effect of isolation procedure on the sedimentation constant of the isolated glycogen. Glycogens isolated from two halves of the same rabbit liver using (*a*) boiling water and (*b*) 30% aqueous potassium hydroxide had almost the same sedimentation constants (relative values 85:86 respectively). Subsequent reprecipitation of a potassium hydroxide extracted glycogen with 80% acetic acid (Bell and Manners, 1952; Manners and Archibald, 1957) similarly had a negligible effect on the value of the sedimentation constant S_{20}. By contrast further digestion of a rabbit liver glycogen in 8% aqueous sodium hydroxide at 100° for 1·5 hr reduced S_{20} from 86 to 57×10^{-13} c.g.s. units and increased the polymolecularity showing that hot dilute alkali degrades glycogen rapidly. These results were in agreement with those of two earlier workers (Oakley and Young, 1936) who claimed that glycogens obtained by alkaline extraction or by water extraction followed by precipitation with acetic acid in each case had the same molecular size when determined by osmotic pressure.

Determination of Glycogen

In general, methods used for the determination of glycogen in tissues are preceded by an extraction process either with boiling 30% potassium hydroxide or cold aqueous trichloracetic acid as discussed above. The glycogen precipitated from the extract by means of alcohol can then be assayed by determination of the reducing sugars produced by acid hydrolysis (Good, Kramer and Somogyi, 1933; Cori, 1932) or by direct treatment of the precipitated glycogen with anthrone reagent (Morris, 1948; Seifter, Dayton, Novic and Muntwyler, 1950). The major controversies over which method to choose centre round (*a*) the completeness of the extraction procedure and (*b*) whether the sole carbohydrate present in the precipitate is glycogen. Bloom, Lewis, Schumpert and Shen (1951) found that in the livers of unfasted rats the concentration of the glycogen determined by trichloracetic acid extraction was consistently 85% of that found after

alkali digestion. The trichloracetic acid extractable glycogen of muscle in normal fed rats was 55% of that obtained by alkali extraction. In an alternative procedure Kemp and van Heijningen (1954) claimed that all the glycogen could be brought into solution by grinding the tissue with trichloracetic acid solution and then heating the resulting suspension for 15 min at 100°. In a critical appraisal of the various methods, Carroll, Longley and Roe (1956) found that a boiling 30% potassium hydroxide extract of liver contained material that was not glycogen. This material was dialyzable, anthrone-sensitive, reduced alkaline copper solutions and was therefore a source of error in glycogen methods based upon alkali extraction of tissues. These workers contended that a series of five extractions with 5% trichloracetic acid in a Servall omnimixer at 14,000 r.p.m. sufficed to extract all the glycogen even from livers of fasted rats. Precipitation of the glycogen with 5 volumes of 95% alcohol and direct determination of the glycogen with anthrone was claimed to give a sound reliable method.

Electrophoresis of Glycogen and Related Polysaccharides

Foster, Newton-Hearn and Stacey (1956) have studied a wide range of amylosaccharides by paper ionophoresis in alkaline borate and other buffers. These amylosaccharides were located by immersion of the paper in water–ethanol–conc. hydrochloric acid (10:1:1), and thereafter spraying the dried paper with ethanolic iodine (0·4%). The M_G values obtained (mobility with respect to glucose) in borate buffer pH 10 were D-glucose, 1·00; maltose, 0·36; amylose, 0·18; amylopectin, 0·25; glycogen (rabbit liver), 0·31.

Besides paper, two other supports, glass paper and silk were investigated for use in the separation of polysaccharides (Fuller and Northcote, 1956). A modified *p*-anisidine spray was used to detect the polysaccharides on glass paper. Taking the movement of yeast mannan as 1·0, yeast glycogen had a mobility of 0·45, amylose, 0·38 and amylopectin 0·45 on glass paper in 0·1M-borate buffer, pH 9·3. Other electrophoretic studies of glycogen have been carried out by Bertrand and Laszt (1956) and Geldmacher-Mallinckrodt and Wienland (1953).

Northcote (1954) used a Tiselius electrophoresis apparatus to separate glycogen and other related polysaccharides. In 0·1M-glycine buffer at 0° their mobilities (cm^2V^{-1} $sec^{-1} \times 10^{-5}$) were as follows: yeast glycogen, 0·7, 0·0; rabbit liver glycogen, 1·0; potato starch, 2·2, 0·0; potato amylopectin 1·7, 0·0. As will be seen some of the polysaccharides moved as two components. The mobilities of the polysaccharides in 0·05M-borate buffer, pH 9·2° at 0° were also studied.

A preparative method for the column electrophoresis of polysaccharides including glycogen was described by Hocevar and Northcote (1957). Separation of a mixture of inulin, glycogen, mannan and galactan (each

10 mg) could be accomplished on a column of glass wool (l, 80 cm; diam, 2·5 cm) using 0·05M borate buffer, pH 9·2 as the electrolyte and applying 3V/cm for 9 hr. The fractions were then eluted from the column with more borate buffer and the polysaccharides detected using the anthrone reagent (Seifter, Dayton, Novic and Muntwyler, 1950).

Detection of Glycogen in Tissues

The general basis of histochemical techniques for the detection of glycogen in tissues is (1) the strong reaction it gives in the periodic acid-Schiff (PAS) reaction and (2) the fact that it can be distinguished from other PAS-positive substances by its easy removal with α-amylase. In the application of the periodic acid-Schiff reaction the tissues should be as fresh as possible preferably obtained within one hour of death. Preservation of the tissue can be effected by freeze-drying (Mancini, 1948) followed by a suitable fixative, freeze-substitution (Lisbon, 1949) or acetic-formalin-alcohol (Gendre's fluid). Prior to staining many investigators cover the section with a thin film of celloidin (collodion, nitrocellulose) to keep the glycogen in position. The PAS reaction was introduced into histology by McManus (1946) who recommended exposure for 2 minutes in a 0·5% solution of periodic acid in distilled water. Other variations introduced by Lillie (1947) included periodate acidified by the addition of 0·5% v/v conc. nitric acid, 10 minutes in a solution of 1 gm sodium periodate in 100 ml of 70% nitric acid, 0·69% KIO_4 in 0·3% nitric acid at pH 1·9 for 10 min (Lillie, 1950), and finally a 1% aqueous solution of paraperiodic acid (H_5IO_6) at 20° for 10 min (Lillie, 1954). Hale (1957) in an excellent review of this subject recommends the use of the Hotchkiss alcoholic periodic acid method (Hotchkiss, 1948). This procedure involves 5 minutes' treatment with a solution containing periodic acid (400 mg) dissolved in distilled water, to which is added 5 ml of 0·2M sodium acetate and 35 ml. ethyl alcohol. After exposure to the oxidant the section is washed to remove excess oxidant, exposed to Schiff's solution (leucofuchsin) for 15 to 30 minutes and then further washed in a sulphite solution to remove excess leucofuchsin. A 5-minute wash with water between the oxidant and the Schiff's solution is generally adequate (Hale, 1955). The aldehydic structure of periodate-oxidized glycogen gives a bright magenta colour with Schiff's solution which is basic fuchsin (rosaniline, pararosaniline and magenta II) reduced by the action of sulphite. The subsequent rinse with sulphite prevents false positive reactions resulting from the recolorization of the Schiff's solution by atmospheric oxidation.

Since many other carbohydrate containing components of tissue give a positive PAS reaction it is highly desirable that the identification of glycogen should be checked by prior digestion with α-amylase. In this procedure the tissue is not covered by a celloidin film but incubated directly

with centrifuged saliva at room temperature for one hour. It would perhaps be preferable to use crystalline salivary α-amylase itself since Lillie, Greco and Laskey (1949) report that saliva contains a ribonuclease-like activity.

Sulkin (1960) reports that some mucopolysaccharides which cannot be revealed by the PAS technique can in fact be detected using the technique of Kramer and Windrum (1953; 1954). This is based on the sulphation of neutral mucopolysaccharides in tissue sections which are then visualized by their metrochromatic staining properties following toluidine blue staining.

Molecular Weights and Polydispersity

Molecular weight determinations of glycogens were carried out as early as 1936 by determination of osmotic pressure (Oakley and Young, 1936). The values obtained (rabbit liver glycogen, $1{\cdot}2$–$2{\cdot}2 \times 10^6$; rabbit muscle glycogen, $0{\cdot}7$–$1{\cdot}8 \times 10^6$) were the number average molecular weights. Values of the same order were obtained by Bell, Gutfreund, Cecil and Ogston (1948) using sedimentation and osmotic pressure measurements; horse muscle glycogen, $2{\cdot}9 \times 10^6$; rabbit muscle glycogen, $2{\cdot}6 \times 10^6$; human muscle glycogen, $2{\cdot}4 \times 10^6$; rabbit liver glycogen $4{\cdot}4 \times 10^6$. The glycogen was isolated from each source under alkaline conditions.

Examination of a glycogen in an ultracentrifuge is particularly useful since it reveals any inhomogeneity; it indicates also that the glycogen molecules behave in solution as spheres. Bridgman (1942) found that rabbit liver glycogen exhibited a spread of values from 20 to 120S with the maximum component having a sedimentation constant of 70S. Polglase, Brown and Smith (1952) found that normal human liver glycogen contained two polydisperse components with sedimentation constants in the ranges 60–100S and 150–300S. A glycogen isolated from a liver biopsy specimen obtained from one patient with von Gierke's disease contained only the lighter material. Examination of human muscle glycogens from two normal patients revealed a major heavy component and a minor light component, but from a third normal patient a glycogen with only the heavy component was obtained. Glycogen obtained from a patient having glycogen storage disease of muscle had a much greater proportion (30–40% instead of 10–15%) of the lighter component present irrespective of whether it was obtained at autopsy or biopsy or from skeletal or heart muscle.

Although determination of molecular weights of glycogen by light scattering gives weight-average molecular weights, and thus attributes relatively more significance to the large than to the small molecules in a polydisperse population, this method has found favour with many recent workers particularly in combination with ultracentrifugation studies. The

molecular weights determined by Stetten, Katzen and Stetten (1956) using the light scattering technique have already been mentioned. The method tends to indicate larger particle sizes; values of $1–20 \times 10^6$ being obtained by Putzeys and Verthoeven (1949) and Staudinger (1948).

The method of Isbell (1951) in which the combining weight of a polysaccharide is calculated from the radioactivity of the product of addition of $Na^{14}CN$ with the unique reducing end of each polysaccharide molecule is unsuitable for native glycogen. Meyer's colorimetric method (Meyer, Noelting and Bernfeld, 1947) is only suitable for degraded liver glycogens in the range M.W. < 300,000.

Structural Studies of Glycogen

Products from methylated glycogen

Treatment of glycogen triacetate in acetone solution with a mixture of methyl sulphate and potassium hydroxide afforded a partially methylated glycogen (OMe, 40%). Repeated treatments gave a trimethyl glycogen

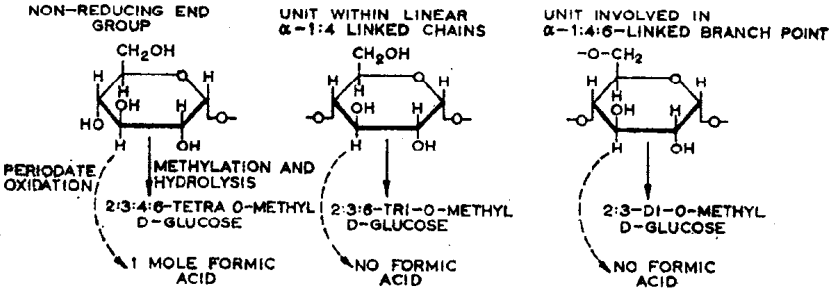

Fig. 1.1a *Glucose units in glycogen*

(OMe, 43·7%; $[\alpha]_D^{20}+208°$ in $CHCl_3$) in 90% yield (Haworth, Hirst and Webb, 1929). When heated with boiling 2% methanolic hydrogen chloride for 24 hr, this trimethyl glycogen afforded a mixture of products from which methyl 2:3:6-tri-*O*-methyl glucoside could be recovered by distillation *in vacuo*. Subsequent aqueous hydrolysis of the latter gave crystalline 2:3:6-tri-*O*-methyl α-D-glucopyranose (see Fig. 1.1a). Haworth and Percival (1931) later degraded trimethyl glycogen with acetyl bromide and oxidized the products with bromine in the presence of barium benzoate. After remethylation and esterification they obtained methyl octamethyl maltobionate which itself yielded crystalline 2:3:4:6-tetra-*O*-methyl D-glucopyranose and 2:3:5:6-tetra-*O*-methyl γ-gluconolactone on aqueous acid hydrolysis. Haworth and Percival (1932) succeeded subsequently in isolating crystalline 2:3:4:6-tetra-*O*-methyl D-glucopyranose from methylated rabbit liver glycogen in a yield (9%) indicative of the

presence of one non-reducing end group for every twelve residues. Glycogens were later investigated in which this ratio was found to be one in every eighteen glucose residues (Bell, 1936; Haworth, Hirst and Isherwood, 1937). The latter workers also isolated an amount of dimethyl glucose equivalent to that of the tetramethyl glucose and suggested a laminated formula for glycogen in which the adjacent chains of α-1:4-linked glucopyranose residues were joined as in Fig. 1.2a. Haworth, Hirst and Smith (1939) found that three specimens of glycogen from dogfish, haddock, and hake liver and one from dogfish muscle contained a repeating unit of 12 α-1:4-linked glucopyranose residues. The introduction of a small scale method for the analysis of mixtures of tetra-, tri- and di-*O*-methyl sugars on silica columns greatly simplified and rendered more reliable the end group assay of methylated polysaccharides (Bell, 1944). Methylated horse muscle glycogen assayed in this way had an average chain length of 12 glucose residues.

The nature of the linkage uniting the chains in glycogen still remained a problem. Although Bell (1948) succeeded in analysing the mixture of dimethyl glucoses (obtained from methylated glycogens) by periodate oxidation the difficulty of ensuring full methylation of the glycogen rendered only approximate his findings that the major dimethyl glucoses were 2:6 and 2:3.

Acid hydrolysis products

From the knowledge that maltose hydrolyses four times faster than *iso*maltose (see Fig. 1.1b) in 2% concentration in 0·050 N-sulphuric acid at 99·5°, Wolfrom, Lassettre and O'Neill (1951) calculated that the maximum yield of *iso*maltose obtainable from the hydrolysis of glycogen was 6·8% and that this maximum occurred when 89% of the glycogen had been destroyed. Rabbit liver glycogen was hydrolysed with 0·05 N-H_2SO_4 at 100° for 8 hr (degree of hydrolysis, 66%) and the hydrolysis products acetylated with sodium acetate/acetic anhydride. Separation of the acetylated hydrolysate on a column of Magnesol-Celite (5:1) afforded crystalline β-D-glucopyranose pentaacetate, β-maltose octaacetate, β-*iso*maltose octaacetate and β-maltotriose hendecaacetate. In later work, Wolfrom and Thompson (1957) fractionated the acid hydrolysate of a beef liver glycogen (69% hydrolysis; 92 g) on a carbon column into disaccharide and trisaccharide fractions. Separation of the acetylated disaccharide mixture on Magnesol-Celite gave crystalline β-maltose octaacetate, β-*iso*maltose octaacetate and β-nigerose (3-*O*-α-D-glucopyranosyl-D-glucopyranose) octaacetate in amounts of 5·2 g; 4·6 g and 2 mg respectively. Separation of the trisaccharide mixture by paper chromatography afforded crystalline panose [*O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose] and amorphous *iso*maltotriose (see Fig. 1.1b).

The structure of the latter was rigidly established by the nature of the partial acid hydrolysis products of the *iso*maltotriose and its reduction product, *iso*maltotriitol. The yields of panose and *iso*maltotriose obtained were 1·05 g and 0·67 g respectively. Maltotriose, isolated as its crystalline hendecaacetate (0·12 g), was also obtained. The above work firmly established that the major branch points in glycogen were constituted by

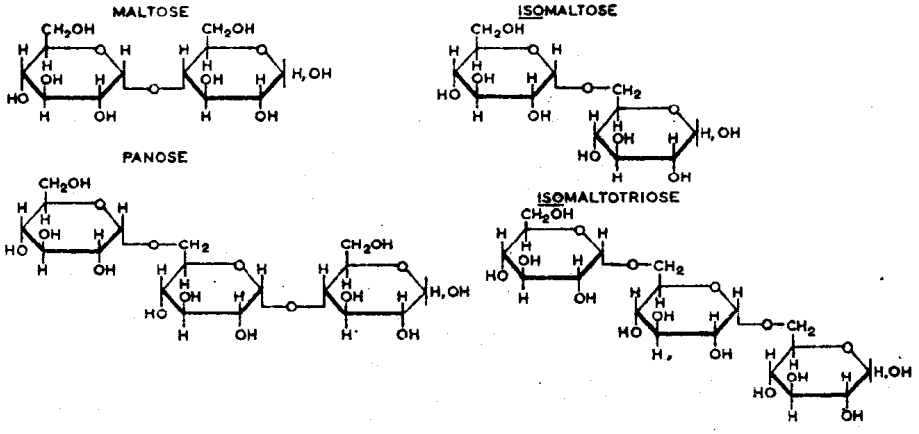

Fig. 1.1b *Acid hydrolysis products of glycogen*

joining the adjacent chains of α-1:4-linked glucopyranose residues with α-1:6-glucosidic linkages and to a very minor extent with α-1:3-glucosidic linkages. The significance of the finding of *iso*maltotriose, which implies that some of the α-1:6-linkages are adjacent, has been neglected by many subsequent workers. The finding of nigerose in such small quantities may be due to its formation via acid reversion of glucose.

Evidence from periodate oxidation

Halsall, Hirst and Jones (1947) applied the technique of periodate oxidation to various glycogens and from the amount of formic acid produced, calculated the number of glucose residues/non-reducing end group since only the latter groups would afford formic acid (see Fig. 1.1a). Oxidation of various glycogens with sodium periodate in the presence of potassium chloride at 20° in dim light gave the following average chain lengths: human muscle, 11; rabbit muscle, 13; horse muscle, 14; guinea-pig liver, 13 and various glycogens from rabbit liver, 14–18. These results were in good agreement with those obtained by the methylation method. Abdel-Akher and Smith (1951) using sodium periodate alone at 5–6° in the dark studied some thirty-seven samples of glycogens from all sources and obtained values from 10–14 including human liver glycogen, 11; rabbit liver, 11–13; horse liver, 11; ox liver, 13; dog liver, 12; and

guinea-pig liver, 10. All the glycogens were highly purified and had $[\alpha]_D$ from +185 to +198° in water. Fifteen samples of glycogen were assayed by Manners and Archibald (1957) using potassium periodate oxidation and twelve of these had average chain lengths of 10–14 glucose residues. However, some exceptions found by Manners and Archibald (1957) and Bell and Manners (1952) include samples of glycogen from human liver, 6 *Helix pomatia*, 7 and from *Mytilus edulis c.* 5, 9, 13 and 17.

Further valuable information as to glycogen structure can be obtained using a method developed by Hirst, Jones and Roudier (1948). The principle involved postulates that in glycogen each glucose unit except those linked at the branch points through either the 1:2:4 or 1:3:4-positions will be oxidized by periodate and on hydrolysis will yield a dialdehyde; if periodate oxidation is complete the presence of glucose in the hydrolysate will indicate interchain linkages of the 1:2 or 1:3-type. Gibbons and Boissonnas (1950) found that with one glycogen the ratio of the number of interchain linkages at C_2 or C_3 to those at C_6 was not greater than 1:42. Bell and Manners (1954) found that a hydrolysate of periodate-oxidized cat glycogen contained no glucose. Abdel-Akher, Hamilton, Montgomery and Smith (1952) applied a variation of this technique which involved acid hydrolysis of the hydrogenated periodate-oxidized glycogen. One sample of glycogen so treated yielded 1% glucose, suggesting that not all the branch points were of the 1:4:6-type. The value of this last variation of the application of periodate oxidation is that determination of the nature and amount of the other products of hydrolysis (glycerol, erythritol, glycollic aldehyde) provide a method of end-group assay and confirm the 1:4 linkages within the chains.

Action of β-amylase

The enzyme β-amylase can be obtained from wheat (Meyer, Spohr and Fischer, 1953), barley (Meyer, Fischer and Piquet, 1951), soya beans (Peat, Pirt and Whelan, 1952) sweet potatoes (Balls, Walden and Thompson, 1948) and other sources. The action of all types of β-amylase involves attack from the non-reducing ends on the exterior chains of the α-1:4-linked glucose residues in amylopectin or glycogen until the enzyme action is obstructed by the interchain α-1:6-linkages. This process, termed β-amylolysis, results in the liberation of maltose from these exterior chains and leaves a 'β-limit' dextrin of high molecular weight with only two or three glucose residues projecting beyond each branch point. Some of the branching end-group arrangements which have proved resistant to β-amylase are shown in Fig. 1.1c (French, 1960). The determination of the percentage of maltose liberated affords a measure of the exterior chain length in glycogens and amylopectins. The results obtained with certain glycogens are quoted in Table 1.I.

Table 1.I. *Reactions of glycogens with β-amylase*

Glycogen from:	Average chain length	Conversion (%) into maltose	Source of β-amylase
Beef liver[1]	—	45	Wheat
Rabbit liver[2]	—	45	Wheat
Rabbit liver[3]	12	43	Barley
Rabbit liver[4]	13	43	Sweet potato
Rabbit liver I[5]	13	25	Barley
Rabbit liver III[5]	13	51	Barley
Rabbit liver V[5]	14	51	Barley
Rabbit muscle[4]	13	45	Sweet potato
Rabbit muscle II[5]	11	39	Barley
Foetal-sheep liver[4]	13	49	Sweet potato
Cat liver[4]	13	48	Sweet potato
Cat liver IV[5]	13	53	Barley
Cat liver VI[5]	12	52	Barley
Human muscle[4]	12	41	Sweet potato
Human muscle II[5]	11	40	Barley

[1] Meyer and Press (1941); [2] Morris (1944); [3] Northcote (1952); [4] Bell and Manners (1952); [5] Liddle and Manners (1957).

Most glycogens therefore have exterior chain lengths of *ca.* 8 glucose residues and interior chain length of *ca.* 4 glucose residues although quite a large degree of variation exists even in glycogens from the same type of animal tissue.

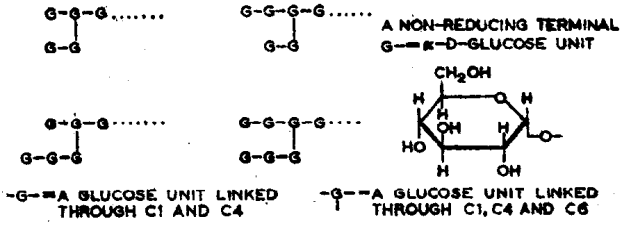

Fig. 1.1c

Action of α-amylase

Human saliva is a rich source of α-amylase (Meyer, Fischer, Staub and Bernfeld, 1948). Whereas the sole products of the action of salivary α-amylase on amylose are maltose and maltotriose (Whelan and Roberts, 1953) those resulting from the incubation of glycogen with the enzyme include in addition a series of branched α-dextrins (Whelan and Roberts, 1952). The α-1:6 linkages present in these α-dextrins are susceptible to

attack by R-enzyme, a 'debranching' enzyme obtained from potatoes and broad-beans (Hobson, Whelan and Peat, 1951). The number of reducing groups liberated during the scission of the α-1:6-linkages in the α-dextrins can be determined and gives yet another method of assessing the average chain length of glycogen.

Roberts and Whelan (1960) have shown that treatment of glycogen with α-amylase yields maltose, maltotriose (ratio 2·03:1) together with α-limit dextrins the smallest of which is a pentasaccharide. No splitting of α-1:6 linkages was detected. A small amount of maltulose (4-*O*-α-D-glucopyranosyl D-fructose) was detected among the products of α-amylolysis of rabbit liver glycogen. High concentrations of salivary α-amylase will hydrolyse maltotriose further to glucose and maltose and a tetrasaccharide can then be detected among the products from the α-amylolysis of glycogen (Walker and Whelan, 1960).

The nature of the α-dextrins obtained from glycogen also provides further evidence as to the fine structure of this polysaccharide. Various

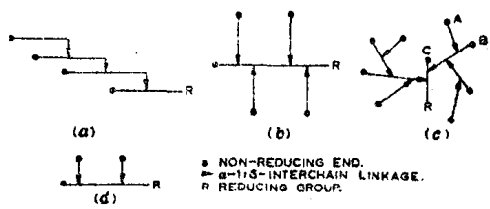

Fig. 1.2

types of branched structures are possible for glycogen among which are a singly branched 'laminated' structure Fig. 1.2a (Haworth, Hirst and Isherwood, 1937), a 'comb-like' structure (Fig. 1.2b) proposed by Freudenberg and the multiply branched 'tree' structure (Fig. 1.2c) of Meyer and Bernfeld (1940). Examination of the α-dextrins (Whelan and Roberts, 1952) revealed that some were doubly branched (Fig. 1.2d) showing that multiple branching occurs in glycogen and favouring a structure of the type shown in Fig. 1.2c. Three types of chains of α-1:4-linked D-glucopyranose residues exist in Fig. 1.2c.

A—is a chain joined to the rest of the molecule only by one α-1:6-linkage.

B—is a chain to which one or more A-chains are attached and which is itself linked to an adjacent chain by an α-1:6-linkage.

C—is the chain terminated by the sole reducing group.

Other evidence for a multiply branched structure in glycogen has been obtained by the stepwise degradation of glycogen with phosphorylase and amylo-1:6-glucosidase (Larner, Illingworth, Cori and Cori, 1952).

Glycogen–Iodine Complexes

When stained with iodine/potassium iodide under the standard conditions used for the determination of blue value (Bourne, Haworth, Macey, and Peat, 1948), glycogens stain only weakly compared with potato amylose (B.V. 1·25), amylopectin (B.V. 0·19) and starch (B.V. 0·41). If, however, the concentrations are altered so that the light absorption is 2·5 times that used in blue value determinations (Peat, Whelan, Hobson and Thomas, 1954) the iodine complex of oyster glycogen is found to exhibit a λ_{max} at *ca.* 480 mμ. Eight samples of glycogen from various sources examined in this way were found to give glycogen–iodine complexes with maximum absorption at 420–470 mμ (Liddle and Manners, 1957). Potato amylose and potato amylopectin give iodine complexes with λ_{max} at 640 mμ and 560 mμ respectively. Absorption at these longer wavelengths thus seems characteristic of the much longer α-1:4 D-glucopyranose chains present in amylose and amylopectin although the exact correlation still remains to be established.

Reaction between Concanavalin-A and Glycogen

Many years ago, Sumner and Howell (1936) discovered that concanavalin-A, a globulin extracted from Jack Bean meal, precipitated glycogen from aqueous solution. This protein–carbohydrate reaction can be measured turbidimetrically and has been used for the determination of glycogen (Cifonelli and Smith, 1955) in the range 0·1 to 1·0 mg/ml. Neither periodate-oxidized glycogen nor the polyalcohol derived therefrom by reduction show any reaction with concanavalin-A. Methylated glycogen likewise shows no precipitating ability (Cifonelli, Montgomery and Smith, 1956). However, removal of the outer chains of glycogen by β-amylolysis actually renders it more readily precipitable by an amount approximately proportional to the degree of hydrolysis. The glycogen value (G.V.), which is the precipitating capacity with respect to a standard rabbit liver glycogen (G.V. = 1·00), varies for glycogens isolated from different sources: ox liver, 0·95; horse liver, 1·05; rat liver, 1·40; human liver (von Gierke's disease), 1·30; human liver (normal), 1·00. Concanavalin-A does not react with amylopectins, waxy corn starch, potato starch, *Leuconostoc mesenteroides* NRRL 512B dextran or laminarin. Concanavalin-A does, however, form an insoluble complex with yeast mannan (Sumner and O'Kane, 1948) while with it heparin (Cifonelli, Montgomery and Smith, 1956) has 50% more precipitating power than normal human liver

glycogen. However, chondroitin sulphate, hyaluronic acid and beef lung galactogen give no precipitation reaction with concanavalin-A.

Reaction of Glycogens with Antipneumococcal Horse Sera

Type II *Pneumococcus* polysaccharide contains D-glucose units involved in 1:4:6-branch points together with L-rhamnose and D-glucuronic acid residues (Butler, Lloyd and Stacey, 1955; Butler and Stacey, 1955). Type II antipneumococcus sera cross-reacts with glycogens from human liver, dog liver and oysters (Heidelberger, Aisenberg and Hassid, 1954), tamarind seed polysaccharide (Heidelberger and Adams, 1956), dextrans (Heidelberger and Aisenberg, 1953) and other polysaccharides (e.g. amylopectin) containing α-1:4:6-linked D-glucose units. Similar cross reactions are shown by Types VII, IX, XI, XVIII, XX and XXII antipneumococcal horse sera.

Enzymic Synthesis of Glycogen

Phosphorylases

The first evidence concerning the mechanism of synthesis of glycogen was found by Cori, Colowick and Cori (1937; 1938a) and Cori, Schmidt and Cori (1939) using a dialyzed muscle extract. These workers established the reversibility of the reaction whereby a salt of α-D-glucopyranose 1

$$n.\ C_6H_{11}O_5\ .\ O\ .\ PO_3K_2 \rightleftharpoons (C_6H_{10}O_5)_n + nK_2HPO_4 \qquad (1)$$

(dihydrogen phosphate) was converted by muscle phosphorylase *in vitro* to a polysaccharide giving an intense blue stain and resembling amylose the unbranched α-1:4-glucosan. Later Hassid, Cori and McCready (1943) demonstrated that muscle phosphorylase required the presence of a primer such as glycogen before it would exhibit any synthetic activity. Rabbit skeletal muscle phosphorylase was isolated in the crystalline form by Green and Cori (1943) and found to have a molecular weight of 340,000–400,000. This enzyme was designated phosphorylase *a* and showed 60–70% of its full activity without addition of adenylic acid. The solubility of the enzyme was greatly increased in weak salt solutions by the addition of cysteine. A more soluble protein, phosphorylase *b* was also present in rabbit muscle but was inactive without added adenylic acid (Cori and Green, 1943). In the presence of adenylic acid both forms of the enzyme had the same activity per mg of protein. The American workers also isolated another enzyme (PR) from muscle or spleen which when incubated with phosphorylase *a* converted it to phosphorylase *b*. The same conversion could be achieved by incubation with crystalline trypsin at pH 6 (Cori and Cori, 1945). The PR enzyme had very low activity in the absence of cysteine