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Recent Developments in Our Understanding of Glycogen Structure

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ABSTRACT

The main structural features of glycogen were established many years ago by predominantly chemical methods. However, the fine structure continues to be the subject of experimental investigations by enzymic methods. Current topics include: (a) the mechanism of the formation of the linkage between glycogen and protein during the first stages of biosynthesis; (b) the arrangement of the constituent chains to form β-particles of molecular weight ~ 10^7; (c) the mode of aggregation of β-particles to form much larger α-particles; and (d) the possible presence of monosaccharide residues other than D*-glucose, and of phosphate ester groups. This review comprises a critical discussion of the above and related aspects of glycogen structure.*

INTRODUCTION

Although the main structural features of glycogen, the reserve carbohydrate of most animal cells, have been known for many years, research interest in this polysaccharide shows no signs of diminishing. There are several reasons for this. Firstly, the detailed fine structure is continuing to be the subject of investigation and speculation, in contrast to the impression given by certain textbooks. Secondly, the physical form of the molecules, which may occur as particles with molecular weights of ~ 10^7 (β-particles), or as very much larger particles (α-particles) is still being studied, as is the relationship between the β- and α-particles. Thirdly, it is now known that glycogen from some animal tissues is, in fact, a glycoprotein, (or more correctly, a proteoglucan) rather than a pure poly-

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saccharide. The nature of the glycogen–protein linkage formed during the first stages of biosynthesis has been established, but the enzymic mechanism for the initiation of glycogen synthesis is not yet completely understood. Fourthly, recent researches have shown that some highly purified glycogen preparations contain significant amounts of monosaccharides other than D-glucose, and also phosphate ester groups, this latter being contrary to long-held beliefs. Fifthly, it is now recognised that in liver and certain other tissues, glycogen is metabolically heterogeneous, and exists in both the cytosol and the lysosomes. The two forms differ in molecular weight and protein content, so that the 'tertiary' structure of the glycogen molecules in a particular tissue may be different, even though they are synthesised to the same general macromolecular pattern. Finally, improvements in experimental techniques have been so great that glycogen from a wide range of tissues having a low glycogen content can now be examined, e.g. from brain tissue containing only about 0·03% of glycogen; biopsy samples from cases of glycogen storage disease can also be investigated.

This review will concentrate on the above aspects of glycogen structure. Attention is drawn to a number of other reviews. The classical structural studies on glycogen have been described by Meyer (1943), Bell (1948*a*) and Manners (1957), whilst Geddes (1985) has reviewed more recent work, particularly from a physico-chemical point of view. The literature on the related subject of glycogen metabolism is very extensive, but will not be considered here, except as far as any structural implications are concerned. Reviews by Stetten and Stetten (1960) and Ryman and Whelan (1971) are recommended. The papers and discussions in the CIBA Foundation Symposium on '*Control of Glycogen Metabolism*' edited by Whelan and Cameron (1964) and a FEBS Symposium with the same title, also edited by Whelan (1968) showed the parallel development of structural and metabolic studies, and posed a number of questions which are still relevant today.

THE BASIC STRUCTURE OF GLYCOGEN

Glycogen is usually described as a high molecular weight polymer (10^7–10^9) of α-D-glucose whose aqueous solution is opalescent, has a negligible reducing power, a high dextrorotation of about $+196°$ and gives a red-brown stain with iodine. The classical chemical studies showed that the macromolecules were composed of linear chains normally containing an average of 10–14 (1→4)-linked α-D-glucose residues, which were interlinked by (1→6)-α-D-glucosidic linkages, to

form a branched structure. The average chain length was initially determined by methylation analysis as being either 12 or 18 glucose residues (Bell, 1948*a*), but later periodate oxidation analyses gave results ranging from 10 to 18, with most in the range 10 to 14, (Halsall *et al.*, 1947). All of these results were obtained prior to 1950, and were confirmed in later periodate oxidation analyses, in which glycogen from several dozen different sources was examined (Abdel-Akher & Smith, 1951; Bell & Manners, 1952; Manners & Archibald, 1957; Kjolberg *et al.*, 1963). The degree of branching in glycogen is therefore about twice that in amylopectin, which usually has an average chain length ranging from 20 to 25. Several properties of glycogen and amylopectin are compared in Table 1.

The nature of the (1→6)-α-D-glucosidic inter-chain linkages was deduced from the presence of some 2,3-di-*O*-methyl-D-glucose amongst the hydrolysis products of methylated glycogen (Haworth *et al.*, 1937) and the isolation of small amounts of isomaltose from a partial acid hydrolysate (Wolfrom *et al.*, 1951). However, Bell (1948*b*) reported that 2,6-di-*O*-methyl-D-glucose was the major di-*O*-methyl sugar in hydrolysates of methylated rabbit muscle and liver glycogen, and suggested the possibility of some (1→3) inter-chain linkages. By contrast, a later methylation study of glycogen polyalcohol (i.e. after periodate oxidation and borohydride reduction) gave a negligible amount of the 2,6-di-*O*-methyl-D-glucose (Bahl & Smith, 1966).

Other evidence to support the possibility of (1→3) inter-chain linkages at this time was the presence in glycogen of about 1% of

TABLE 1
Properties of Amylopectin and Glycogen-type Polysaccharides[a]

Property	*Amylopectin*	*Glycogen*
General structure	Branched	Highly branched
Average chain length (CL)	20–25	10–14
Degree of polymerisation (DP)	10^5–10^6	~10^5
β-Amylolysis limit, %	~55	~45
Exterior chain length (ECL)	13–16	6–8
Interior chain length (ICL)	6–8	3–5
Ratio A-chains:B-chains	1·0–1·5:1	0·7–1·0:1
Aqueous solution	Stable	Stable
Iodine coloration, λ_{max}, nm	530–550	430–460
Limiting viscosity number [η], ml/g	150–220	5–10
Variation of sedimentation constant with concentration	Dependent	Independent

[a]Based on data from Manners and Sturgeon (1982).

periodate-resistant residues (Abdel-Akher *et al.*, 1952) and a minute quantity of nigerose (0·001%) in a partial acid hydrolysate (Wolfrom & Thompson, 1957). Later work showed that incomplete periodate oxidation and acid-catalysed transglucosidation were responsible for the above results (Bell & Manners, 1954; Manners *et al.*, 1965). In reality, there was no unambiguous evidence for any inter-chain linkages other than the (1→6)-α-D-glucosidic type. This means that the structure and metabolism of glycogen can be considered in terms of about 90% of (1→4)-α-D-glucosidic linkages and about 10% of (1→6)-α-D-glucosidic linkages.

During the 1930s and 1940s, glycogen was usually isolated from tissues by one of four methods. The classical method introduced by Bernard and Pflüger involved heating the tissue with hot strong alkali (30% potassium hydroxide was often used), followed by precipitation with ethanol. Other cellular constituents (proteins, nucleic acids) would be destroyed by this drastic treatment. Alternatively, tissues such as liver, with a high glycogen content, (about 5% by weight) could be chopped into small pieces and extracted with boiling water, followed by deproteinisation with 4% trichloroacetic acid (TCA) at 0°C (Bell & Young, 1934) or directly extracted with ice-cold 5–10% trichloroacetic acid. Further purification was effected by repeated precipitation by 80% acetic acid and/or ethanol. Meyer (1943) used 33% aqueous chloral hydrate at 80°C and pH 6–7 to extract glycogen from mussels, followed by deproteinisation with picric acid.

All of these methods had some limitations. The Pflüger method could result in limited alkaline degradation of the glycogen. Several workers reported that the sedimentation coefficients of glycogens extracted by this method, and by hot water, were very similar (e.g. Bryce *et al.*, 1958), but later work has shown that the *distribution* of sedimentation coefficients is greatly affected (Geddes, 1985). Moreover, TCA-extracted glycogen could also be degraded by hot 30% potassium hydroxide (Stetten *et al.*, 1958). In the case of hot water and TCA-solvents the extraction of glycogen was incomplete, and led to a debate on the significance of 'free' and 'bound' glycogen, and the binding of glycogen to protein in the cell (Stetten & Stetten, 1960).

For these and other reasons, newer isolation methods have been devised using milder solvents such as dimethylsulphoxide and 0·2 M glycine buffer (pH 10·4). These will be discussed later in relation to the proteoglucan nature of glycogen and the isolation of β- and α-particles. It should be noted that some workers have used commercial preparations of glycogen for their work. The actual method of isolation may therefore be unknown, and there is evidence that such samples have

lower molecular weights than laboratory-prepared samples. In this laboratory, carefully prepared samples, in which the extracting solvent was known, have been used, and adventitious contamination with either saliva or acid was avoided.

By the 1940s, three different molecular models for glycogen had been proposed. Haworth *et al.* (1937) suggested a laminated or singly branched structure to account for the difference between the average chain length (CL) of about 12, and the degree of polymerisation (DP) of about 3000–5000 (see Fig. 1). Staudinger and Husemann (1937) proposed an alternative structure to account for the methylation results, and the evidence from viscosity measurements that the molecule was almost spherical. They suggested that glycogen consisted of a central chain of up to about 100 (1→4)-linked α-D-glucose residues, to which

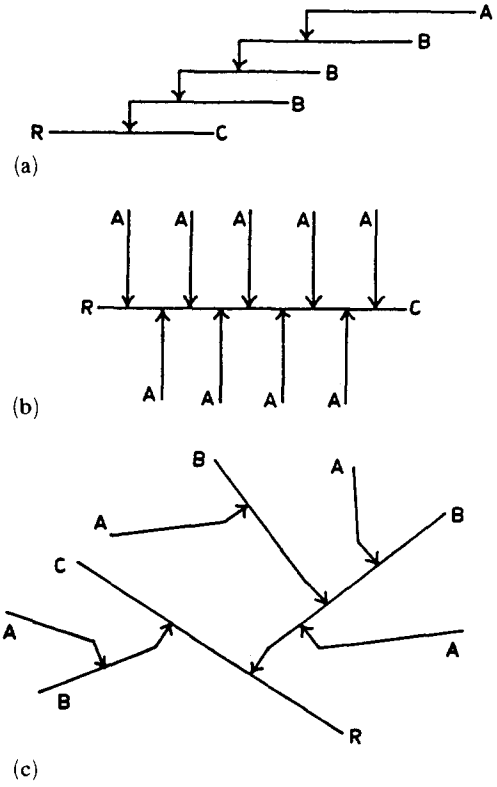

Fig. 1. Molecular structures for glycogen. (a) Haworth 'laminated' form; (b) Staudinger 'comb' form; and (c) Meyer 'tree' form. ——, Linear chain of (1→4)-α-linked D-glucose residues; ↓, inter-chain linkage ((1→6)-glucosidic in structures (a) and (c); (1→2), (1→3), and (1→6)-glucosidic in (b)); A, B and C, are types of chain (see p. 43) R, the potential reducing group. From Manners (1957), reproduced by permission of Academic Press.

was attached at C2, C3 and C6 of each residue a linear side chain of about 12 (1→4)-linked α-D-glucose residues. Methylation analysis would in effect measure the length of the side chains, but would yield D-glucose rather than di-*O*-methyl-D-glucose on hydrolysis of the methylated polysaccharide.

The third structure by Meyer and Fuld (1941) was based on one of the first enzymic analyses of glycogen. A commercial sample (Merck) of mussel glycogen with a CL of 11 by methylation analysis, was treated with wheat β-amylase giving 47% of maltose and 53% of a residual dextrin. Methylation analysis showed the latter to have a CL of 5·5. β-Amylolysis was confined to the exterior chains of glycogen and on the assumption that the 'stubs' remaining after enzyme action contained an average of 1·5 glucose residues, the authors concluded that the exterior chains contained about seven glucose residues and the interior chains about three glucose residues (see Fig. 1(c)). On the basis of this information, the authors postulated a multiply branched tree structure for glycogen, although the methylation results do not, in fact, enable a distinction to be made between singly and multiply branched structures. The suggestion was probably based on an analogy to a related analysis of amylopectin, where treatment of a β-limit dextrin with a yeast glucosidase preparation (of unknown purity and uncertain specificity) led to the formation of a second limit dextrin which could then be further partially degraded by β-amylase to give a second β-limit dextrin (Meyer & Bernfeld, 1940). If a singly branched structure was subjected to this sequential enzymic degradation, then the second β-amylolysis would result in the *complete* degradation of the substrate. In retrospect, the proposal of a multiply branched structure for glycogen, which is now known to be correct, can be viewed as an inspired suggestion, since the original paper by Meyer and Fuld (1941) does not describe any enzymic degradation beyond the initial β-amylolysis.

The first unambiguous evidence of multiple branching in glycogen was provided by Larner *et al.* (1952) using a stepwise degradation by muscle phosphorylase and amylo-(1→6)-glucosidase. Their overall conclusions have been confirmed by workers in other laboratories. These and related experiments will be considered in some detail after a discussion of the structural parameters of glycogen, and of enzymic methods of analysis.

THE STRUCTURAL PARAMETERS OF GLYCOGEN

These parameters are similar to those used to describe amylopectin-type molecules (Manners, 1989*a*) and for convenience, are repeated below.

Exterior chains are those parts of a chain between the non-reducing end-group and the outermost branch point, whilst interior chains represent parts of a chain between branch points in the interior of the molecule (see Fig. 2). A-Chains are linked to the molecule only by the potential reducing group, whilst B-chains are similarly linked, but also carry one or more other chains. The C-chain carries the sole potential reducing group in the molecule, although for some purposes, the possible presence of a reducing group is not significant, and the C-chain can be regarded as a B-chain. (In many glycogen samples, this reducing group is covalently linked to an amino-acid residue in a protein 'backbone' (see p. 57).) The concept of A-, B- and C-chains was introduced by Peat *et al.* (1952*b*, 1956) and the degree of multiple branching may be expressed as the ratio of A-chains to B-chains.

It must be emphasised that analytical methods for measurement of the chain length (CL), exterior chain length (ECL) and interior chain length (ICL) give average values, and that individual chains can vary considerably in length. From the above definitions and Fig. 2 it can be seen that a glycogen molecule contains virtually equal numbers of exterior and interior chains. If the molecule has an A:B-chain ratio of 1:1, then each A-chain is also an exterior chain, and each B-chain, on the average, consists of one exterior and two interior chains. In fact, many B-chains are now known to consist of one exterior chain and only one interior

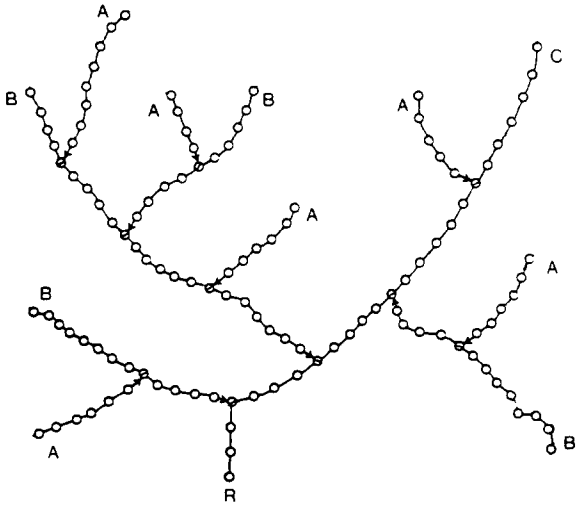

Fig. 2. Segment of a hypothetical branched (1→4)-α-D-glucan. In glycogens, the exterior chains may contain 6–8 glucose residues, whereas in amylopectins, they are about twice this length. ○—, A (1→4)-linked α-D-glucose residue; ⊘←, a (1→6)-linked α-D-glucose residue; A, B and C, types of chain; R, a potential reducing group.

chain. Other B-chains contain one exterior chain and three or more interior chains. The proportion of very long B-chains will decrease as their CL increases. Nevertheless, there is now evidence (see p. 50) for a small proportion of B-chains containing perhaps 50 or more glucose residues which play a key role in the overall molecular structure.

If a glycogen molecule had an A:B-chain ratio of 0·7:1, the basic assumptions made above remain, but the quantitative aspects require minor adjustments.

THE ACTION PATTERN OF GLYCOGEN DEGRADING ENZYMES

Although the basic structure of glycogen was investigated by chemical methods, much of the later work has involved the use of highly purified enzymes of known specificity. The action pattern of starch degrading enzymes, which also degrade glycogen, has been reviewed previously (Manners, 1989*a*). A brief summary now follows.

The incomplete degradation of a glycogen, of known chain length, by β-amylase, to give $45 \pm 5\%$ of maltose and a β-limit dextrin, can be used to determine the relative lengths of the exterior and interior chains. The size of the 'stubs' remaining in the degraded exterior chains must be known. The action of purified muscle phosphorylase is also confined to the exterior chains and many glycogens give 30–35% of glucose 1-phosphate and a phosphorylase-limit dextrin (ϕ-dextrin) which is now known to contain maltotetraose 'stubs' (Walker & Whelan, 1960). The extensive fragmentation of glycogen by α-amylase to give maltose, a series of branched α-dextrins and small amounts of glucose and/or maltotriose can be used as a method for estimating the average chain length (Manners & Wright, 1962), whilst analysis of the structure of the α-dextrins has confirmed the nature of the inter-chain linkages (Roberts & Whelan, 1960).

The most useful amylolytic enzymes for structural analysis are the debranching enzymes, which selectively hydrolyse the $(1\rightarrow6)$-α-D-glucosidic inter-chain linkages. Historically, the first of these to be characterised was rabbit muscle amylo-$(1\rightarrow6)$-glucosidase which released glucose from glycogen ϕ-dextrin, and enabled further phosphorolysis to proceed (Cori & Larner, 1951). Originally, it was believed that the ϕ-dextrin had side chains (A-chains) consisting of one glucose residue, and main chain (B-chain) 'stubs' with 5–6 glucose residues. Later work (Walker & Whelan, 1960) showed that both the A- and B-chain 'stubs' in a ϕ-dextrin contained four glucose residues.

Amylo-(1→6)-glucosidase has a dual action, in firstly transferring a maltotriose residue from an A-chain to a B-chain, and then hydrolysing the single glucose residue that remained as the A-chain 'stub'. The combined action of muscle phosphorylase and amylo-(1→6)-glucosidase resulted in the complete degradation of glycogen to give ~90% of glucose 1-phosphate and ~10% of glucose. Determination of the latter enabled the chain length to be measured (Illingworth *et al.*, 1952). The stepwise degradation of glycogen by phosphorylase and amylo-(1→6)-glucosidase has provided firm evidence of a multiply branched structure (see p. 60).

Debranching enzymes from bacterial sources have been of even greater value in structural analyses. Pullulanase from *Aerobacter aerogenes* (*Klebsiella aerogenes*) caused partial debranching of many samples of glycogen and glycogen β-dextrin, as shown by increases in β-amylolysis limit and iodine-staining power. It is assumed that enzyme action involves the preferential release of the A-chains, but the enzyme cannot penetrate into the interior of the molecule. By contrast, isoamylase from *Pseudomonas* and *Cytophaga* catalyses the complete debranching of glycogen to give a heterogeneous mixture of linear chains (Akai *et al.*, 1971; Gunja-Smith *et al.*, 1970*a*). The reducing power liberated during this hydrolysis can be used to determine the average chain length (Gunja-Smith *et al.*, 1971), whilst the distribution of the size of the liberated chains, as revealed by gel filtration, shows the chain profile (Gunja-Smith *et al.*, 1970*b*). Yeast isoamylase will also debranch glycogen, but not as completely as the bacterial enzymes, and has therefore been of lesser value, although the limited action on glycogens from uncommon sources has enabled their outermost inter-chain linkages to be identified.

Plant debranching enzymes have not have been used in the structural analysis of glycogens. Broad bean R-enzyme (limit dextrinase) had little or no action on glycogen of normal chain length, i.e. about 12, although it would debranch α-dextrins produced by the α-amylolysis of the glycogen (Peat *et al.*, 1954). By contrast, amylopectin, which has a much lower degree of branching, is a substrate.

THE ENZYMIC ANALYSIS OF GLYCOGEN

Determination of average chain length (CL)

Several methods for the determination of the CL of glycogens have been developed, including the use of amylo-(1→6)-glucosidase and

phosphorylase (Cori & Larner, 1951), α-amylase (Manners & Wright, 1962), β-amylase and pullulanase (Lee & Whelan, 1966) and β-amylase with amylo-(1→6)-glucosidase (Carter & Lee, 1971). All of these methods could be used to analyse glycogens on the milligram scale, and gave results in good agreement with those obtained by methylation or periodate oxidation analysis of the *same* sample. However, all have now been superceded by the use of bacterial isoamylase, which causes complete debranching of the macromolecule (for a review, see Harada, 1989).

The increase in reducing power is related to the number of reducing groups liberated on debranching, and can be used on as little as 200 μg of glycogen (Gunja-Smith *et al.*, 1971). The method can be applied equally to glycogens and amylopectins and is in routine use in many laboratories.

Determination of exterior and interior chain lengths

The use of β-amylase to determine the ECL and ICL of mussel glycogen (Meyer & Fuld, 1941) has been described on p. 42. For complete accuracy, the method requires an exact knowledge of the size of the 'stubs' remaining on the exterior chains of the β-dextrin. Meyer and Fuld (1941) assumed the 'stubs' contained an average of 1·5 glucose residues, so that ECL = number of glucose residues removed by β-amylase + 1·5. However, Peat *et al.* (1952*b*) showed that the A-chain 'stubs' in amylopectin β-dextrin contained two or three glucose residues, since on debranching, maltose or maltotriose was released. On the assumption that the B-chain 'stubs' had a similar length, ECL was then calculated from the number of glucose residues removed by β-amylase + 2·5 (e.g. Liddle & Manners, 1957). Shortly afterwards, French (1960) deduced that the B-chain 'stubs' contained either one or two glucose residues. If glycogen contained approximately equal numbers of A- and B-chains, then ECL would be more correctly given by ECL = number of glucose residues removed by β-amylase + 2·0. Literature values published prior to 1960, and indeed, others published after this date, may be in error by 0·5 glucose residue. In view of possible errors in the determination of CL (± 1 glucose residue) and β-amylolysis limit (± 2%), the error may not be serious, except where ICL values of less than three have been reported (which implies an incredible degree of branching in the interior of the molecule), or where authors unrealistically reported CL values to within 0·1 glucose residues. Somewhat surprisingly, Geddes (1985) has used the 'stub' value of + 2·5 in his discussion.

A further source of error relates to the actual branch-point residue. In their original work, Meyer and Fuld (1941) excluded this from the ECL and ICL so that CL = ECL + ICL + 1. This equation has been used by many other workers, including the present author and his coworkers. However, Geddes (1985) stated that ECL values normally included the branching residue, whilst others have used the equation CL = ECL + ICL without specifying the location of the branch-point residue. In this review, the original interpretation of Meyer and Fuld (1941) will continue to be used.

One experimental point should be emphasised. In view of the very large difference in molecular weight between glycogen α-particles and β-particles, it is important to note that the extent of β-amylolysis is independent of molecular weight (Geddes, 1985), provided of course, that an excess of β-amylase is present in the enzyme digests.

In the overall determination of ECL, it is assumed that all the exterior chains, i.e. the A-chains and the exterior portions of the B-chains, are of similar lengths. Experimental proof of the correctness of this assumption has been available in the literature for some years, but has not received special comment. The experiments involved treatment of glycogen with pullulanase, which liberated the A-chains; these were fractionated by gel filtration. The average CL of the products was in the range 6·0 to 8·0, which was in accord with the calculated ECL. The products were a range of maltosaccharides with CL values varying from two or three to more than nine, showing that the A-chains varied considerably in length. The experiments were carried out on rabbit liver and oyster glycogens (Akai *et al.*, 1971), on shellfish glycogen (Hata *et al.*, 1983) and on oyster glycogen (Misaki & Tsunoda, 1984). The most elegant demonstration of the size and distribution of A-chains is shown later in Fig. 10, from the work of Misaki and Yano (1985), on oyster glycogen. These results are of special significance, since one recent molecular model for glycogen included A-chains which were very much longer than the outer portions of the B-chains (Goldsmith *et al.*, 1982; see p. 66), and is therefore incorrect.

Determination of A:B chain ratio

Although the A:B chain ratio in amylopectins has been examined in several laboratories (for reviews see Manners, 1985, 1989*a*, *b*), this important ratio in glycogen has attracted less attention. The first experiments on amylopectin involved debranching of the β-limit dextrin, to give either maltose or maltotriose, the yield of which was proportional to the number of A-chains (Peat *et al.*, 1952*b*, 1956). These workers used a

broad bean debranching enzyme (R-enzyme) which had no action on glycogen, so that in the first experiments on glycogen, pullulanese had to be used (Bathgate & Manners, 1966). The results with several samples of glycogen β-dextrin indicated a high proportion of A-chains that either approached or was equal to that calculated for multiply branched molecules containing equal numbers of A- and B-chains. Recent recalculation of the results (Manners, 1989*b*) gave a range of values from 0·6:1 to 0·9:1 for five samples and 1·1:1 for a sixth (Table 2).

In an alternative method, Akai *et al.* (1971) measured the yield of A-chains liberated from glycogen by the action of pullulanase. The calculated A:B-chain ratios were 1:1·0 for rabbit liver glycogen and 1:1·1 for oyster glycogen.

More recently, Umeki and Yamamoto (1977) reported A:B chain ratios of 0·67:1 for shellfish glycogen and 1·3:1 for glutinous rice starch (amylopectin) based on the debranching of the β-dextrins with pullulanase. For glycogen, their results are based on the belief that 45% of the maltotriose arose from the B-chains. This fact implies that within a glycogen β-dextrin, the partial structures shown in Fig. 3 were present. This novel aspect of glycogen fine structure requires further investigation, especially since two sequential treatments of oyster glycogen with pullulanase gave a range of maltosaccharides, the smallest of which was maltotetraose (Misaki & Yano, 1985). Maltotriose, which would have been expected from Fig. 3, was absent.

TABLE 2
Properties of Some Glycogens and Amylopectins[a]

Sample	*CL*	*β-Amylolysis limit (%)*	*ECL*	*ICL*	*A:B-Chain ratio*
Glycogens					
Ascaris lumbricoides	12	49	8	3	0·6:1
Human liver	15	46	9	5	0·8:1
Mytilus edulis	13	46	8	4	0·9:1
Oyster	10	40	6	3	0·7:1
Pig liver	15	50	9–10	4–5	1·1:1
Rabbit liver	13	50	8–9	3–4	0·7:1
Amylopectins					
Potato amylopectin	24	55	15	8	1·3:1
Waxy sorghum	24	58	16	7	1·2:1
Waxy maize	20	50	12	7	1·0:1

[a]Based on Manners (1989*b*).

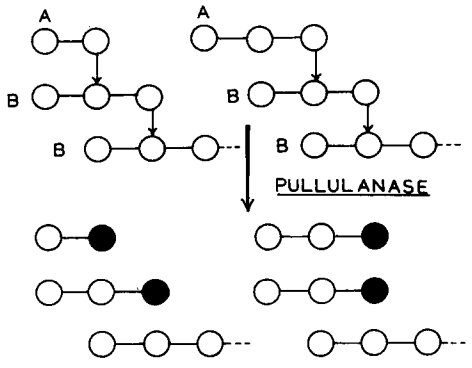

Fig. 3. Hypothetical structures in glycogen β-limit dextrin showing the release of maltotriose from a B-chain. ●, Newly formed reducing groups.

In an alternative method for the determination of the A:B chain ratio, Marshall and Whelan (1974) measured the reducing power liberated when glycogen β-dextrin or amylopectin β-dextrin was digested with (a) isoamylase alone or (b) isoamylase and pullulanase. Since isoamylase was believed to have no action on maltosyl stubs in a β-dextrin, the difference in reducing power between (b) and (a) was equivalent to half of the A-chains. For eight samples of amylopectin, the method gave a range of values from 1·5:1 to 2·6:1. In five samples the ratio was $>2{\cdot}0:1$, which was an apparently novel structural feature. For seven samples of glycogen, the ratios varied from 0·6:1 to 1·2:1, and indicated a significant difference between the two classes of polysaccharide. However, the method is subject to certain errors, and the conclusion that amylopectins have an A:B chain ratio of about 2:1 is no longer justified (Manners & Matheson, 1981; Manners, 1985). With appropriate corrections, waxy maize starch had a ratio of 1·0:1, and glycogen from *Helix pomatia* and rabbit liver gave values of 0·6:1 and 0·8:1 respectively (Manners & Matheson, 1981).

The absolute values for the seven samples of glycogen examined by Marshall and Whelan (1974) are not known, but the overall trend would indicate a difference from amylopectin.

In the case of amylopectins, the results from several laboratories show that in most samples, there are rather more A-chains than B-chains, with the ratios varying from about 1·0:1 to 1·5:1, (Manners, 1989*a*). The reverse is now apparent for glycogens, where many samples contain more B-chains than A-chains. This difference has implications for the molecular models for the polysaccharides.

Examination of chain profile

The complete debranching of an amylopectin or glycogen by isoamylase, followed by gel filtration, enables the chain profile to be revealed. In the first experiments, using *Cytophaga* isoamylase, rabbit liver glycogen gave a symmetrical curve with a single peak corresponding to a DP of 14, which was similar to the average chain length of that sample. Individual chains ranged in DP from about 3 to 50 (Gunja-Smith *et al.*, 1970*b*). Similar results were obtained by Akai *et al.* (1971), using *Pseudomonas* isoamylase. Rabbit liver glycogen gave a single curve with a peak at DP 15, whilst with oyster glycogen, the peak was at DP 11. The CL of these glycogens, as determined by periodate oxidation, was 14 and 12 respectively. Each curve showed a broad distribution of chains from DP 3 to about 50. These chain profiles for glycogen were different to those from amylopectin, which show polymodal distributions (Manners, 1989*a*).

Mammalian glycogens have a greater iodine-staining power than invertebrate glycogens. The aqueous solutions of the glycogen–iodine complexes show λ_{max} values of 450–490 nm and 420–440 nm respectively (Archibald *et al.*, 1961). An explanation for this difference has been provided by examination of the chain profiles of rabbit muscle and *Cardium tuberculatum* glycogens (Craig *et al.*, 1988). The former contained a significant proportion of chains with a DP > 60, whereas none were present in the invertebrate glycogen (see Fig. 4). The interior chains of the two glycogens were very similar, as shown by iodine-staining analysis of their β-dextrins, but the muscle glycogen contained a small number of exterior chains which were very much longer than the average value of 7–8 glucose residues. These longer exterior chains, as part of the B-chains, could bind iodine more firmly, possibly by helical entrapment of the iodine, and hence give a stronger iodine coloration.

All these chain profiles are based on data plotted on a weight basis (i.e. μg or mg polysaccharide/ml). Palmer *et al.* (1983*a*) have suggested that elution data should be plotted on a numerical basis (i.e. μmol of chains/ml). The weight-based profile of oyster glycogen showed marked asymmetry (Fig. 5(a)). The numerically based profile was asymmetrical, with a preponderance of long-chain material (Fig. 5(b)). This profile had a peak at DP 8 (whereas CL was 12), and between DP 8 and 45, it was almost the shape of an exponential curve, with a slight excess of chains of DP 15–25. The weight- and numerically based profile of rabbit liver glycogen (CL 14, see Fig. 5(c)) gave similar results. In both glycogens, there was an almost exponential relationship between the number of component chains and DP over the range DP 8–53. This exponential relationship was consistent with a spherical multiply branched structure.

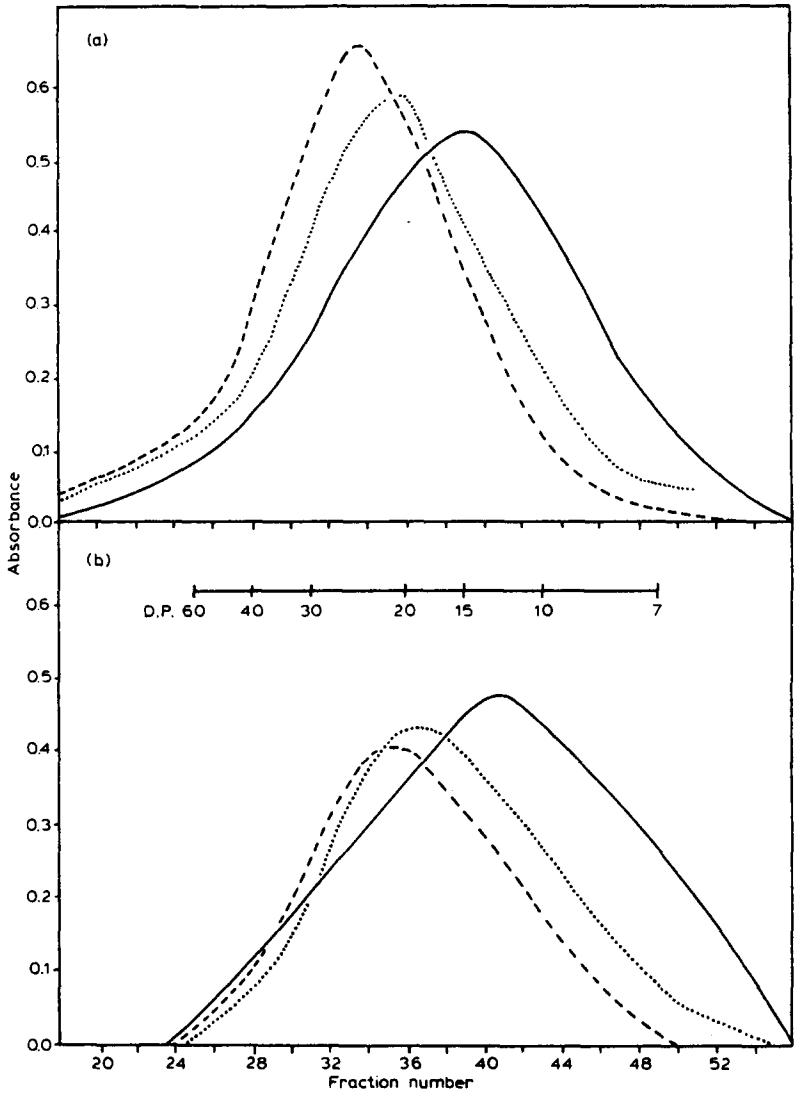

Fig. 4. Molecular weight distribution curves for (a) mammalian (rabbit muscle) and (b) invertebrate (*Cardium tuberculatum*) debranched glycogens by gel chromatography on Sephadex G50. ——, Total carbohydrate; ...., absorbance of iodine complex at 450 nm and – – –, absorbance of iodine complex at 500 nm. From Craig *et al.* (1988), reproduced by permission of Elsevier, Amsterdam.

By contrast, amylopectins which showed two distinct chain profiles by weight, also gave two chain populations on a numerical basis (see Fig. 5). This analysis reveals a significant difference between the two types of polysaccharide.

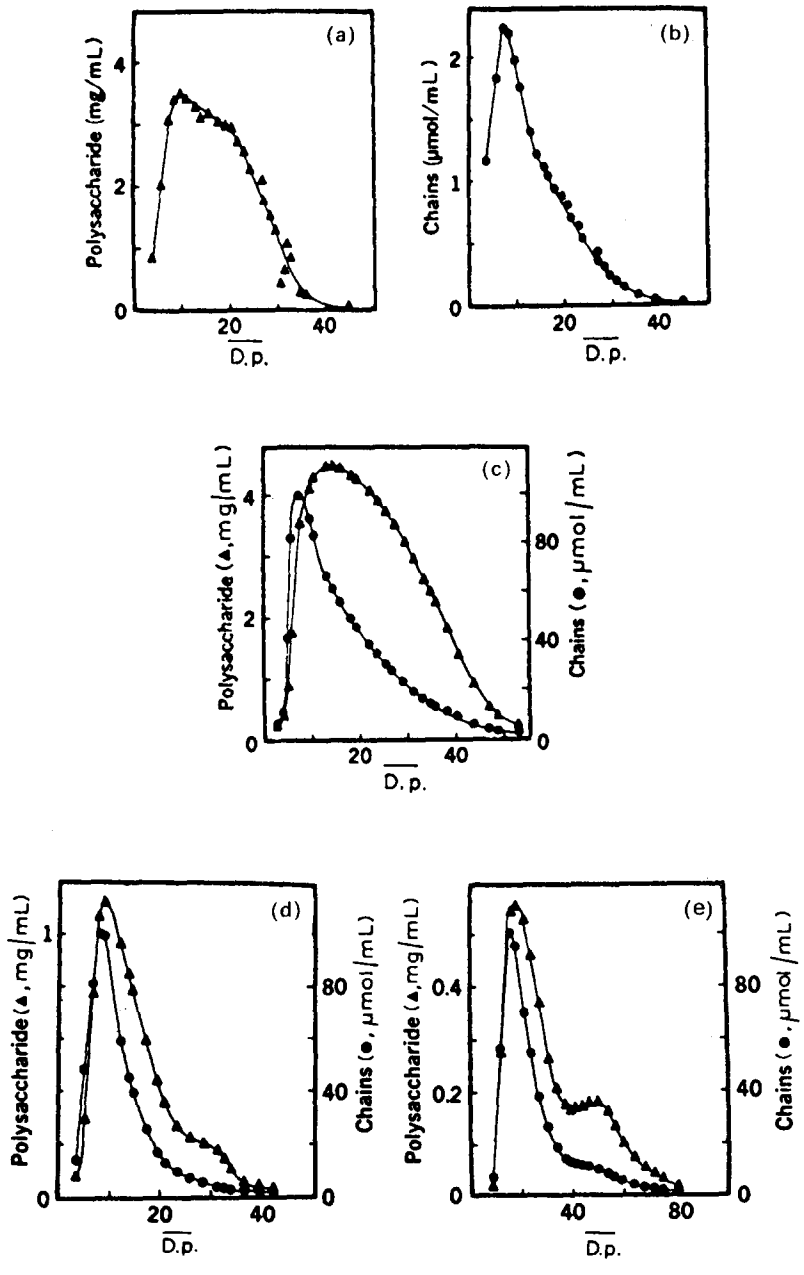

Fig. 5. Unit-chain distribution profiles of (a) oyster glycogen, μg/ml; (b) oyster glycogen, μmol/ml; (c) rabbit-liver glycogen, ▲, μg/ml and ●, μmol/ml; (d) sweet corn phytoglycogen, ▲, μg/ml and ●, μmol/ml; (e) waxy maize amylopectin, ▲, μg/ml and ●, μmol/ml. In (c), (d) and (e), the numerical plots are normalised, the peak fraction being arbitrarily assigned a value of 100. ($\overline{DP}$ = Average degree of polymerisation) from Palmer *et al.* (1983*a*), reproduced by permission of Elsevier, Amsterdam.

THE ENZYMIC SYNTHESIS OF GLYCOGEN

Before considering the detailed fine structure of glycogen, a brief consideration of the biosynthesis of the macromolecule is required. The (1→4)-α-D-glucosidic linkage arises from a chain-lengthening reaction, catalysed by glycogen synthase, which in animal tissues, repetitively transfers α-D-glucosyl residues from uridine diphosphate glucose (UDPG) to an existing chain of (1→4)-linked α-D-glucose residues $[G]_x$:

$$\text{UDPG} + [G]_x \rightarrow G\text{-}(1\rightarrow4)\text{-}[G]_x + \text{UDP}$$

In bacterial tissues, adenosine diphosphate glucose (ADPG) is the α-D-glucosyl donor. The resultant linear chains are converted into branched chains by branching enzyme, which in mammalian tissues, transfers chain segments of DP 6–7, from one chain to the same or an adjacent chain. In most glycogen-synthesising tissues, there appears to be an almost constant ratio of activity between the branching enzyme and glycogen synthase, since the degree of branching is fairly constant. For example, in a survey of 84 different glycogens, 62 had CL values in the range 11–13 (Manners, 1968).

The specificity of glycogen synthase merits comment, since the enzyme can tolerate some variation in the monosaccharide residue of the nucleoside diphosphate sugar substrate. UDP-D-xylose and UDP-D-glucosamine can serve as glycosyl donors with some mammalian cell preparations (Kimura & Caplan, 1978; Kirkman & Whelan, 1986), whilst yeast glycogen synthase can incorporate 2-deoxy-D-glucose (dGlc) from UDPdGlc (Zemek *et al.*, 1971). Reports that some samples of glycogen contain small amounts of D-fructose (Peat *et al.*, 1952*a*) or D-galactose (Nordin & Hansen, 1963) have biosynthetic implications which have not been clarified.

The acceptor specificity of glycogen synthase is very relevant to the molecular structure of glycogen. When singly branched α-dextrins were examined as acceptors, the rabbit muscle enzyme selectively transferred single glucose residues to the B-chain (main chain) and not to the A-chain (side chain). Enzyme action involved a multichain mechanism (Brown *et al.*, 1965). When glycogen or glycogen phosphorylase-limit dextrins (φ-dextrins) were acceptors, the enzyme extended some chains more than others. Although the evidence was not as clear cut as with the α-dextrins, it appeared that B-chains (main chains) were elongated more readily than A-chains (side chains). Enzymic transfer was therefore asymmetrical. If elongation of the B-chains was greater than A-chains, then the subsequent action of branching enzyme would involve transfer of a maltohexaose or maltoheptaose unit from the exterior part of a

B-chain, and the transferred segment would then become an A-chain in the resultant molecule. Further examination of this problem would be worthwhile, since at present it appears that there could be some restriction to a random and fully three-dimensional polymerisation process.

The specificity of branching enzyme is also very relevant to the structure of glycogen. The liver enzyme acted upon glycogens whose exterior chain length exceeded six glucose residues, and was rapid when the exterior chains contained about 11 glucose residues (Larner, 1953). The enzyme detached a maltoheptaose unit (Verhue & Hers, 1966) which could then be attached to the same chain, or to a neighbouring chain (see Fig. 6). In a glycogen macromolecule, with literally thousands of exterior chains, the final A:B ratio will be related to the mechanism of action of the branching enzyme.

The enzymic synthesis of glycogen as a homopolysaccharide of D-glucose was investigated during the 1950s and 1960s, and by 1969, the in vitro synthesis of high molecular weight particulate glycogen using rat liver glycogen synthase and branching enzyme had been achieved (Parodi *et al.*, 1969). This particulate glycogen was very similar to native glycogen with respect to iodine-staining power, sedimentation coefficient in sucrose gradients, and the effect of acid and alkali on the molecular size. However, the question of the in vivo primer for glycogen synthase had not been settled.

The classical studies from Leloir's laboratory showed that glycogen, glycogen ϕ-dextrin, glycogen β-dextrin, maltotetraose and higher maltosaccharides would function as primers (Leloir *et al.*, 1959; Goldemberg, 1962). If a maltosaccharide of DP >4 was to function *in vivo*, then an enzyme system for the biosynthesis of maltotetraose would be required. There was also the possibility of de novo synthesis, particularly with bacterial glycogen synthase (Fox *et al.*, 1973), but the experimental evidence for this was equivocal, in view of the near impossibility of ensuring that the enzyme preparation was entirely free of maltosaccharide impurities.

The eventual solution to the identity of the primer proved to be quite different, and to involve a protein as the primer molecule. Krisman and Barengo (1975) reported that a high-speed pellet from rat liver catalysed the incorporation of glucose from ^{14}C-labelled UDPG to give a glycogen-like product which was precipitated by TCA. The precipitate was solubilised by incubation with pronase. In a control experiment with glycogen added as a primer, the ^{14}C-labelled product was not precipitated by TCA. A hypothesis to explain these results is shown in Fig. 7. It was suggested that four proteins were involved. A protein primer served as an acceptor for glucose residues from UDPG, which were

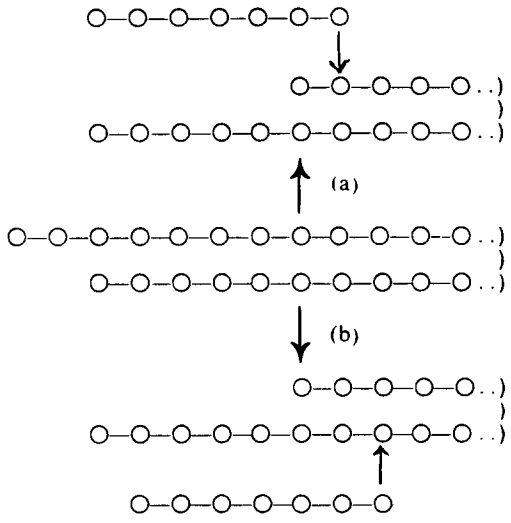

Fig. 6. Enzymic transfer of a maltoheptaose unit (a) to the donor chain and (b) to an adjacent chain by a branching enzyme. From Manners (1968).

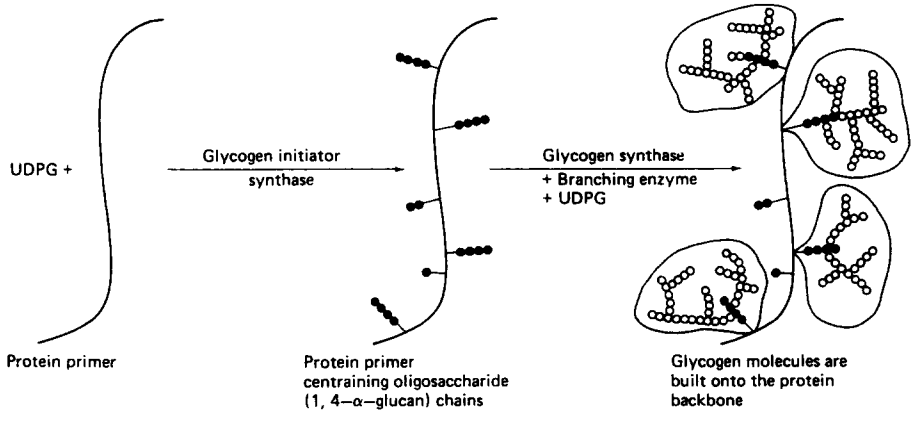

Fig. 7. Glycogen synthesis on a protein primer. The proposed sequence of events in glycogen synthesis is that glucose residues from UDPG (●) are first added to the protein primer by glycogen initiator synthase to form malto-saccharide ((1→4)-α-glucan) chains that will serve as the primers for glycogen synthesis proper, catalysed by glycogen synthase and branching enzyme in the second reaction, where glucose residues for polymerisation are again derived from UDPG (○). (Some of the chains formed by glycogen-initiator synthase have not become long enough to act as primer.) The final product is one in which individual glycogen molecules, with their boundaries shown as a contour, remain covalently attached to the protein primer backbone, forming a giant macromolecule. Redrawn from Krisman and Barengo (1975) by Whelan (1976) and reproduced by permission of Elsevier, Cambridge.

attached covalently as short maltosaccharide chains. This reaction was catalysed by a 'glycogen initiator synthase'. The glycoprotein product of this reaction then served as a primer for glycogen synthase and branching enzyme, so that a number of glycogen molecules were built onto the same protein backbone. This original work was carried out with rat liver preparations, and has been repeated in other laboratories with various tissues, e.g. rabbit skeletal muscle, rat cardiac muscle, bovine retina, *Escherichia coli* and *Neurospora crassa*, giving similar results (for references see Blumenfeld & Krisman, 1986). It is now generally accepted that glycogen biosynthesis in many animal tissues is initiated by a protein primer. This important finding has led to similar conclusions with respect to starch biosynthesis and to a re-examination of glycogen for possible protein components.

GLYCOGEN AS A PROTEOGLUCAN

Although the presence of protein in impure preparations of glycogen was known for many decades, the classical view that glycogen was a true polysaccharide held until the 1970s. Any detection of protein-nitrogen in a glycogen preparation was taken as evidence of a contaminating impurity. A previous review (Manners, 1957) based on the original work of Meyer and Bell and their collaborators stated 'carefully purified preparations of glycogen are free from significant amounts of inorganic material, nitrogen and phosphorus'. Both of the two latter elements are now known to be components of many glycogens. The earlier analytical work was particularly difficult with muscle glycogen, where much of the polysaccharide was protein-bound, and could not be isolated in good yield unless the drastic Pflüger conditions were used.

Following the work of Krisman and Barengo (1975), a re-examination of native glycogen for covalently bound protein was carried out. Butler *et al.* (1977) described evidence for protein-bound glycogen in rat liver. A trichloroacetic acid-insoluble glycogen preparation was subjected to proteolysis, giving a 'glycogen–peptide' fraction. Subsequent purification and detailed analysis of this fraction showed a firm association between the carbohydrate and the peptide. A similar examination of rabbit skeletal muscle provided evidence of glycogen-bound protein (Lomako *et al.*, 1979; Kennedy *et al.*, 1985). Further fractionation studies eventually led to a glycogen containing only 0·35% protein, which could not be decreased. The protein was isolated by treating the glycogen with a

mixture of α-amylase and glucoamylase, and named glycogenin; it had a molecular weight of 37 000. The amount of protein corresponded to one mole of protein per glycogen molecule of molecular weight 10×10^6, which represented the size of a typical β-particle.

Geddes and his coworkers have independently obtained evidence for the proteoglucan nature of both liver and muscle glycogen (for a review, see Geddes, 1985). Their work involved measurement of the size distribution of various glycogens by sucrose density centrifugation, before and after treatment with β-mercaptoethanol and iodoacetamide. This treatment had a profound effect on the distribution of sedimentation coefficients, which could be explained by the disruption of disulphide bonds (although other explanations are possible, see p. 71). This would imply that the individual glycogen molecules (β-particles) are synthesised onto protein backbones, which are held together by disulphide bonds, to form the much larger α-particles. Calder and Geddes (1986) have also examined the effect of proteinase K on both rat muscle and liver glycogens. This enzymic action converted 50–70% of the high molecular weight species (mol. wt $> 250 \times 10^6$) into low molecular weight species, and confirmed the structural parallel between muscle and liver glycogen.

The determination of the chemical nature of the glycogen–protein linkage was extremely difficult, and after several years of endeavour, the identification of a novel glucose–tyrosine linkage by Whelan and his collaborators represented a major achievement in glycogen biochemistry. The experimental work involved degradation of rabbit muscle glycogen with glucoamylase giving 90–95% of glucose and a carbohydrate 'core' attached to protein (Rodriguez & Whelan, 1985). This was then digested with pronase; amino-acid analysis showed the proportion of tyrosine was greatly enriched over the original glycogen. However, this tyrosine could not be iodinated or nitrated (with tetranitromethane) but could be *N*-methylated. These results show that in the pronase-treated 'core' all the tyrosine was involved in acid-labile glycosidic linkages via their hydroxyl groups.

In an independent study, Aon and Curtino (1985) examined glycogen from bovine retina and also concluded that tyrosine was involved in the covalent linkage to carbohydrate. It is intriguing to note that tyrosine was present in the linkage region and claimed to be iodinated! This finding is the opposite to that of Rodriguez and Whelan (1985).

Events on this area have continued to move quickly. Smythe *et al.* (1988) have isolated a peptide from glycogenin which contained a glucosylated tyrosine residue and have determined the amino-acid sequence of 21 amino-acid residues in its vicinity. In the original studies

of Rodriguez and Whelan (1985), it was reported that two tyrosine residues in glycogenin could not be iodinated or nitrated. However, Smythe *et al.* (1988) found only one glucosylated tyrosine per glycogenin molecule, which they noted was consistent with the finding that each muscle glycogen β-particle contained one molecule of glycogenin (Kennedy *et al.*, 1985). More recently, the complete amino-acid sequence of rabbit muscle glycogenin has been reported (Campbell & Cohen, 1989). The protein consisted of 332 amino-acid residues with a molecular mass of 37 278. The novel glucose–tyrosine linkage occurred at a single site, tyrosine-194.

In related studies, Pitcher *et al.* (1987, 1988) have shown that glycogenin and glycogen synthase exist as a 1:1 complex in rabbit skeletal muscle, and that two distinct enzymic activities were involved in the first stages of glycogen synthesis. The first stage was the glucosylation of the tyrosine-194 residue of glycogenin. This resembled the glycogen initiator synthase reaction postulated by Krisman and Barengo (1985), but involved the transfer of a *single* glucose residue at this site. The second stage was the transfer of about five further glucose residues to this site from UDPG, by glycogenin itself, in an autoglucosylation reaction. Glycogenin behaved as a glucosyl transferase, and required Mg^{2+} or Mn^{2+} for activity. The third stage was elongation of the maltohexaose side chain on glycogenin by glycogen synthase. The glycogenin–glycogen synthase complex was believed to dissociate during glycogen synthesis, so that chain elongation and branching could be continued by glycogen synthase and branching enzyme acting together. One implication of this work is that the maximum number of glycogen molecules in a cell may be determined by the number of glycogenin molecules which were sufficiently glucosylated as to serve as substrates for glycogen synthase (Smythe *et al.*, 1988).

The regulation of the activity of glycogen synthase by phosphorylation/dephosphorylation and by hormones is a major biochemical subject, which cannot be considered in detail in this survey. For recent reviews, Preiss and Walsh (1981), Larner (1986, 1990) and Cohen (1988) should be consulted.

It is significant that several other polysaccharides are now known to be synthesised by a mechanism involving initiation on a protein primer. Examples include potato starch, paramylon (a (1→3)-β-D-glucan) from *Euglena gracilis*, a (1→2)-β-D-glucan from *Agrobacterium*, and dextran from *Streptococcus mutans* and *Leuconostoc mesenteroides* (for references, see Blumenfeld & Krisman, 1986).

STRUCTURAL MODELS FOR GLYCOGEN

Isolation of native glycogen

Before discussing the various recent molecular models for glycogen, and the α- and β-particles, it is necessary to briefly consider the isolation of glycogen from cells and tissues. If the solvent and extraction conditions are too mild, then extraction will be incomplete and the product will not be fully representative of the native glycogen, and will consist essentially of the so-called 'free' or 'lyo-glycogen'. However, if more severe conditions are used, there is always the danger that any α-particles and the macromolecule itself will be degraded, as is now known to be the case when hot alkali is used. The literature contains several methods for the efficient extraction of glycogen, with minimal degradation. These include the following solvents: 3% mercuric chloride solution (Mordoh *et al.*, 1966), dimethylsulphoxide (Whistler & BeMiller, 1962), 45% aqueous phenol solution (Laskov & Margoliash, 1963) and various buffers such as 0·2 M glycine buffer pH 10·4 with chloroform (Orrell & Bueding, 1958) or 0·1 M phthalate buffer pH 4·8 (Drochmans & Dantan, 1968).

In some cases, the extraction of the tissue has to be repeated several times. Further procedures are required for the removal of the extraction solvent, denatured protein and nucleic acid impurities, and can involve prolonged ultracentrifugation, including the use of sucrose density gradients. It must be emphasised that post-mortem glycogenolysis of tissues is a rapid process so that speed is essential in the preliminary stages. The distribution of sedimentation coefficients for glycogen prepared by these methods was quite different to those from TCA- or alkali-extracted samples, and indicated a wide range of molecular sizes with sedimentation coefficients of more than 2000 S, equivalent to molecular weights of $\sim 10^9$. For further discussion of this complex problem, the original references in the review by Geddes (1985), who has made notable advances in this area, and the work of Lutkic *et al.* (1980) should be consulted.

Recent structural models for glycogen

Although the enzymic degradation studies of Meyer and Fuld (1941) led to the postulation of a multiply branched 'tree' or bush-like structure for glycogen (see Fig. 8(a)), the first real evidence of multiple branching was

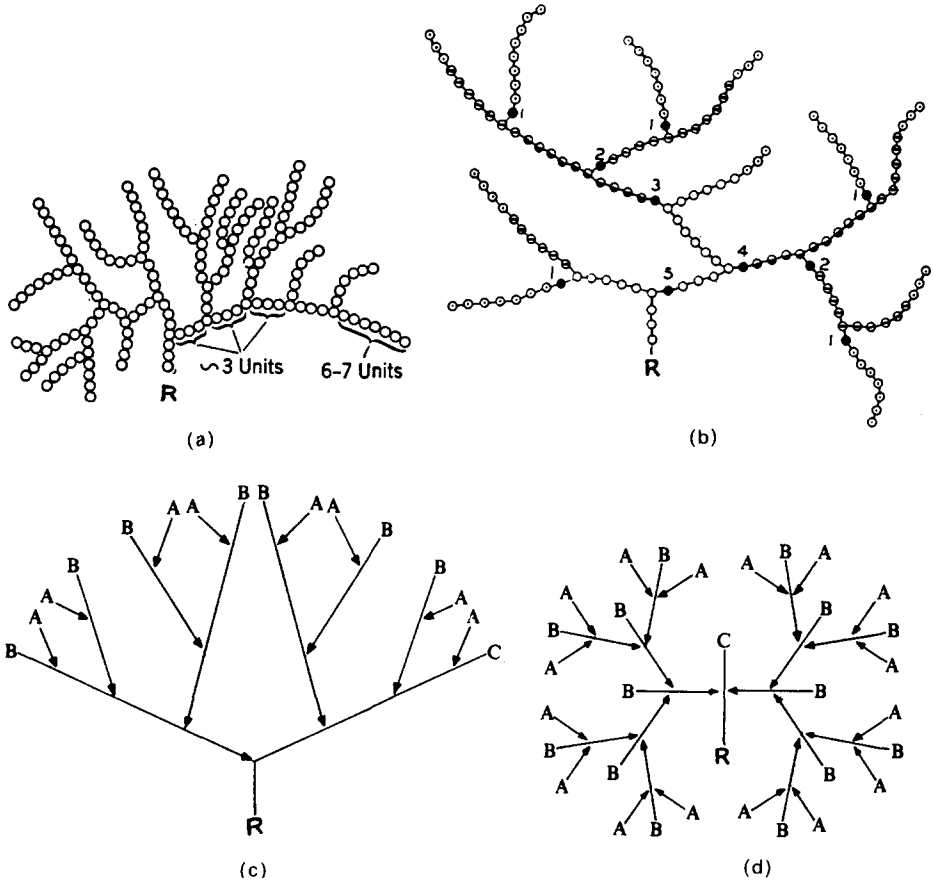

Fig. 8. Molecular models for glycogen. (a) Meyer model as described by Meyer (1943); (b) model of segment of muscle glycogen, based on results obtained by stepwise enzymic degradation by Larner *et al.* (1952) (⊙, ⊖ and ◒, glucose residues removed by first, second and third degradation with phosphorylase, respectively; ●, glucose residues removed by amylo-(1→6)-glucosidase. Of five tiers, three were degraded, corresponding to 122 out of 150 glucose residues); (c) Meyer model redrawn as a regularly rebranched structure, following Lee *et al.* (1968); (d) revised Meyer model, usually referred to as the Whelan model, from Gunja-Smith *et al.* (1970*b*). In all the diagrams, which are reproduced with appropriate permission, R is the potential reducing group.

reported by Larner *et al.* (1952). They carried out stepwise and successive degradations of glycogens by muscle phosphorylase and amylo-(1→6)-glucosidase. The initial phosphorolysis gave about 35% of glucose 1-phosphate and a phosphorylase-limit dextrin (ϕ-dextrin) which, after digestion with amylo-(1→6)-glucosidase, was susceptible to further phosphorolysis. The resultant ϕ-dextrin 2 was again treated with

the debranching enzyme and then phosphorylase, to give a final ϕ-dextrin 3, which amounted to about 12% of the original glycogen. The overall results supported a multiply branched tree-like structure as shown in Fig. 8(b), with the branch points arranged in tiers. The molecular weight of glycogen has been related to the number of tiers in the macromolecule. Madsen and Cori (1958) calculated that for sweet corn phytoglycogen having a CL value of 11·9 glucose residues and an ECL of 7 glucose residues, a molecule with a molecular weight of 20×10^6 would contain about 13 tiers of branch points. D. Stetten in M. R. Stetten and Katzen (1961) calculated that the maximum molecular weight of glycogen was 138×10^6 on the assumption that 17 tiers of branch points were present. The actual maximum size of the molecule becomes limited by the space available at the periphery of the molecule. This is shown diagrammatically in Fig. 9, from the review by French (1964). His calculations indicated a maximum molecular weight of about 20×10^6 for a spherical molecule, and suggested that larger molecules might contain chains which terminated in the interior, i.e. 'buried chains'; these would not be accessible to the action of exo-acting enzymes.

Experimental proof for the presence of a large number of tiers in the glycogen macromolecule has been provided by Misaki and Yano (1985). They subjected oyster glycogen to 15 repeated and successive treatments with pullulanase, and examined the quantity and identity of the maltosaccharides released at each treatment. Their results are shown in Fig. 10. They show that the exterior chains comprised a range of chains with DP 3–9, and that in the deep interior of the molecule, the interior chains contained only two or three glucose residues.

Within the tiered structure, the branch points are not evenly distributed. Salivary α-amylolysis of glycogen yielded α-limit dextrins

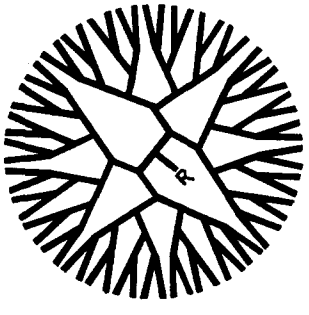

Fig. 9. Space-limited disc structure for glycogen. If each chain is more-or-less regularly rebranched, the periphery of the molecule becomes too crowded to accommodate further branching. From French (1964).

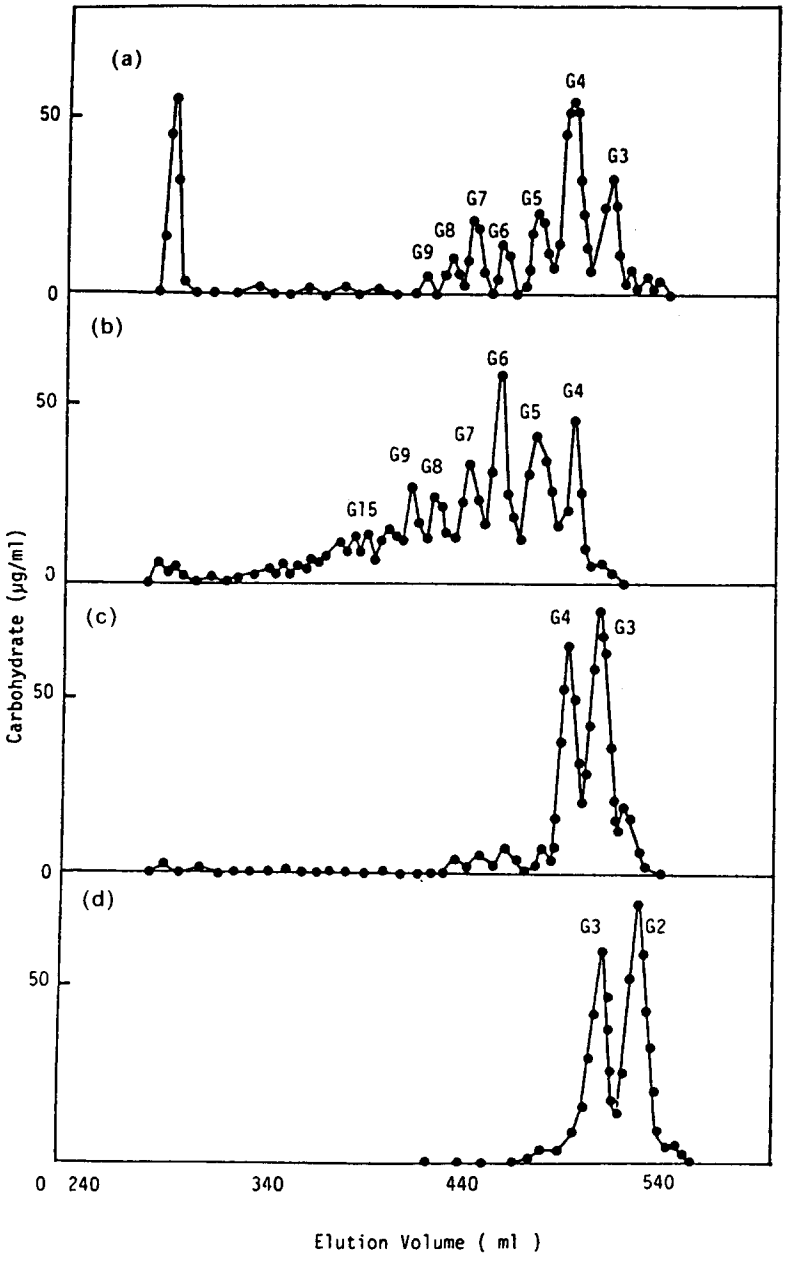

Fig. 10. Gel-filtration profiles of malto-oligosaccharides released by successive debranching actions with pullulanase. (a) 1st Pullulanase digestion. (b) 2nd Pullulanase digestion. (c) 6th Pullulanase digestion. (d) 14th Pullulanase digestion, Column: Bio-Gel P-2 and P-4; Flow rate: 8 ml/h (water). From Misaki and Yano (1985), reproduced by permission of Biryoeiyouso-Kenkyukai, Japan.

containing one, two and perhaps three branch points (Roberts & Whelan, 1960). In the larger α-dextrins, the branch points were separated by only one or two glucose residues, and these very short interior chains were resistant to α-amylolysis. In related work, Schramm (1968) and Brammer *et al.* (1972) have isolated highly branched and relatively high molecular weight 'α-macrodextrins' from α-amylolytic digestions of glycogen. The yield of α-macrodextrin from shellfish glycogen was 11·7% and much greater than that from rabbit liver glycogen (2·4%) and phytoglycogen (1·1%). The α-macrodextrins from rabbit liver and shellfish glycogens had CL values of about 4, and a typical structure is shown in Fig. 11(a) (Schramm, 1968). Brammer *et al.* (1972) showed that shellfish glycogen gave α-macrodextrins with a

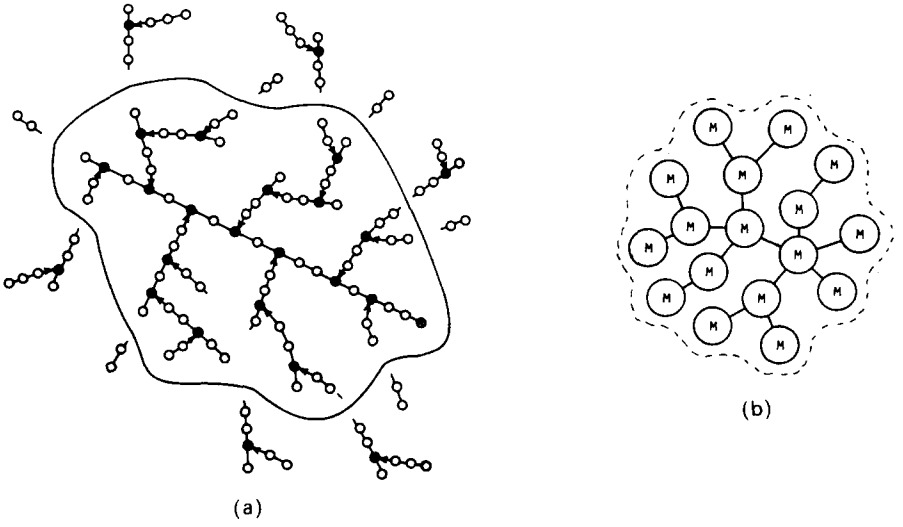

Fig. 11. (a) Illustration of an α-macrodextrin. ○, Glucosyl residues linked only by (1→4)-α linkages; ●, glucosyl residues which bear a side chain (arrow) on carbon 6; ⊗, reducing end of the α-macrodextrin. The points of action of α-amylase which delineate the α-macrodextrin are marked by a solid line. From Schramm (1968). (b) Schematic two-dimensional representation of shellfish glycogen β-dextrin, mol. wt 410 000 (equivalent to mol. wt 760 000 of the original glycogen). The diagram may be regarded as a section of an actual polysaccharide molecule, the entire particle being many times larger than the section indicated. The regions labelled M are densely branched, and on α-amylase action are converted into macrodextrins. Organisation is such that α-amylase initially cleaves the molecule into large fragments, subsequently breaking these down to macrodextrins and oligosaccharides. The reducing group of the original molecule might be attached to any one of the M groups, not necessarily at the centre. The dotted outline represents the periphery of the original glycogen molecule. From Brammer *et al.* (1972), reproduced by permission of Elsevier, Amsterdam.

molecular weight of about 6000, in a 25% yield, and that these macrodextrin regions were randomly dispersed throughout the glycogen molecule (see Fig. 11(b)). They calculated that 77 macrodextrin regions were present per glycogen molecule of molecular weight $3{\cdot}4 \times 10^6$.

In spite of this convincing evidence for an irregular distribution of branch points in the macromolecule, other workers have described glycogen in terms of a regularly rebranched molecule. Lee *et al.* (1968) described amylopectin in these terms, even though amylopectin also yields significant amounts of multiply branched α-dextrins (Roberts & Whelan, 1960). The same diagram (see Fig. 8(c)) was used by Gunja-Smith *et al.* (1970*b*) to describe both glycogen and amylopectin. However, there is no real evidence in the literature to support *regular* branching, even though various analytical techniques give *average* values for CL, ECL and ICL which could be interpreted too literally.

The redrawn Meyer structure for glycogen shown in Fig. 8(c) was revised by Gunja-Smith *et al.* (1970*b*) following the enzymic analysis of the corresponding $\phi\beta$-dextrin, i.e. the β-amylase-limit dextrin of the ϕ-dextrin. At this time, it was believed that *Cytophaga* isoamylase could not hydrolyse maltosyl side chains (A-chains). Hence, treatment of a $\phi\beta$-dextrin based on the redrawn Meyer structure with isoamylase would not release any A-chains, and the debranched $\phi\beta$-dextrin would not be hydrolysed by β-amylase. However, the products from shellfish glycogen and waxy maize amylopectin had β-amylolysis limits of 44 and 29% respectively. An explanation for these and other results is shown in Fig. 8(d). The model comprised equal numbers of A- and B-chains, arranged so that half of the B-chains carried an average of two A-chains, whilst the other B-chains each carried two B-chains. The model contained a number of buried B-chains (compare p. 61). The overall evidence appeared to be sound, except that no comment was made of the release of a surprisingly large amount of maltotriose by the action of isoamylase on the $\phi\beta$-dextrin (see Fig. 2 in Gunja-Smith *et al.*, 1970*b*). As pointed out by Banks and Greenwood (1975) and Palmer *et al.* (1983*b*), this observation actually invalidates the case for a revised structure, since the maltotriose was, most probably, due to incomplete phosphorolysis of the original ϕ-dextrin. If the latter contained some maltopentaosyl A-chains, instead of only maltotetraosyl A-chains, then β-amylolysis would convert these into maltotriosyl A-chains, which would be hydrolysed by isoamylase with the release of maltotriose. Moreover, the resultant polysaccharide would then be susceptible to β-amylase. It has also been known for some time that *Cytophaga* isoamylase would slowly hydrolyse maltosyl A-chains (Evans *et al.*, 1979). The experimental basis for the revised structure is not therefore entirely unequivocal.

The postulation of molecular models for glycogen (and amylopectin) can lead to misunderstandings, especially if the model is taken too literally. Gunja-Smith *et al.* (1970*b*) state their model 'is intended only to offer certain concepts and is not to be regarded as precisely defining glycogen structure'. The concepts that many B-chains carry more than one A-chain and other B-chains carry more than one B-chain are sound, since they are in accord with the observed A:B-chain ratios. Figure 8(d) shows the model drawn in its most symmetrical form, with only two B-chains attached to the C-chain, although there is no experimental evidence for this particular feature. Bullivant *et al.* (1983) have taken Fig. 8(d) literally in their studies of the alkaline degradation of glycogen and have calculated the changes in molecular weight distribution (a) if each glycogen molecule had the structure shown in Fig. 8(d), and yielded only two fragments during alkaline degradation and (b) each glycogen had a redrawn Meyer structure as in Fig. 8(c), and gave rise to a polydisperse series of products. The experimental results are claimed to support case (a) above. (By coincidence, a glycogen molecule as depicted by Meyer (1943) (see Fig. 8(a)) would also yield two major fragments on alkaline degradation, if that diagram was also taken too literally.) Unfortunately, the redrawn Meyer structure in Fig. 8(c), with regular rebranching and a single long C-chain extending through the whole molecule, represents some degree of scientific licence. It is also worth emphasising that the original Meyer structure was proposed some 10 years or so before A-, B- and C-chains had been defined. To summarise at this stage, there is no convincing evidence that the model of Meyer and Fuld (1941) should be disregarded, although it clearly requires modification since the size and complexity of a glycogen molecule is far greater than originally envisaged.

A related structure for glycogen based on the 'Whelan' model, with several tiers of branch points and a protein core, has been proposed by Goldsmith *et al.* (1982) (see Fig. 12). This structure was partly based on two assumptions. Firstly, that there was a uniform distribution of chain lengths of length 11–14; secondly, that the A-chains were much longer than the exterior portions of the B-chains. Unfortunately, neither of these assumptions is true (see p. 50 and p. 47). The concepts of tiers of branch points (these authors have calculated that a β-particle of molecular weight $\sim 10^7$ could contain 12 tiers of branch points) and a protein core are sound, but the fine detail of the chains shown in Fig. 12(a) is incorrect. The greatest value in this structure probably lies in the calculation of a limiting molecular weight of rabbit muscle glycogen (10×10^6), which is similar to that observed in the electron microscope by Drochmans and Dantan (1968), and the probable stoichiometry if

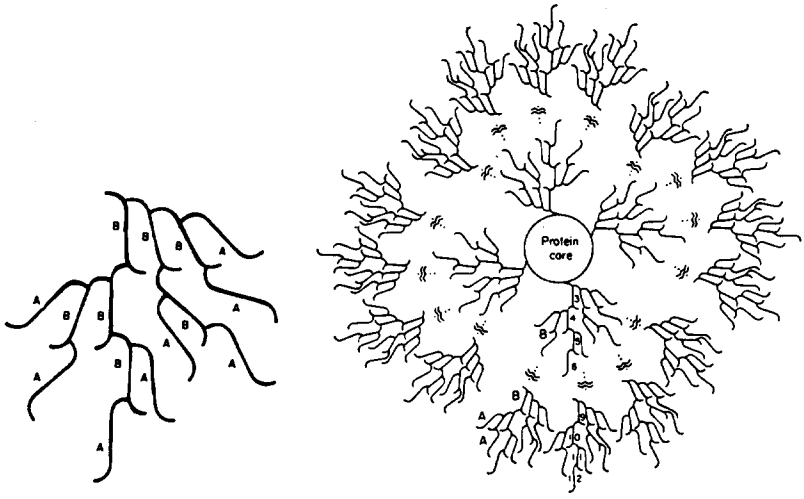

Fig. 12. Schematic diagram of glycogen by Goldsmith *et al.* (1982). (a) Relationship between chains in the Whelan model for four tiers of the structure, and showing A-chains and the exterior portions of B-chains of unequal lengths. (b) Putative protein core with inner and outer tiers of branch points. For clarity, tiers 7 and 8 are missing entirely, and only a fraction of the outer four tiers are shown.

glycogenin of molecular weight 37 000 is present to the extent of 0·35% in glycogen, then one mole of protein requires a glycogen molecule to have a molecular weight of $\sim 10 \times 10^6$ (Kennedy *et al.*, 1985).

Current views on glycogen structure

Any molecular model for glycogen must accommodate the following facts: (a) a very high molecular weight ($\sim 10^7$); (b) an average CL of ~12 with individual chains ranging from about 6 to more than 50 glucose residues; (c) equal numbers of exterior and interior chains; and (d) a ratio of A:B-chains in the range 0·7:1 to 1·0:1. A molecular weight of $\sim 10^7$ implies a molecule containing about 5000 individual chains, so that diagrammatic representation of a whole molecule is not feasible, and only segments of the molecule can be readily shown. For a typical glycogen molecule with CL 13, a β-amylolysis limit of 45%, and an A:B-chain ratio of 1·0:1, each exterior and interior chain will, on the average, contain eight and four glucose residues respectively. Hence, each B-chain which consists, on the average, of one exterior and two interior chains, will contain about 18 glucose residues (see Fig. 13). Whilst the length of individual A-chains does not vary greatly (CL values of three to nine are shown in Fig. 10), B-chains show a wide variation in length. The

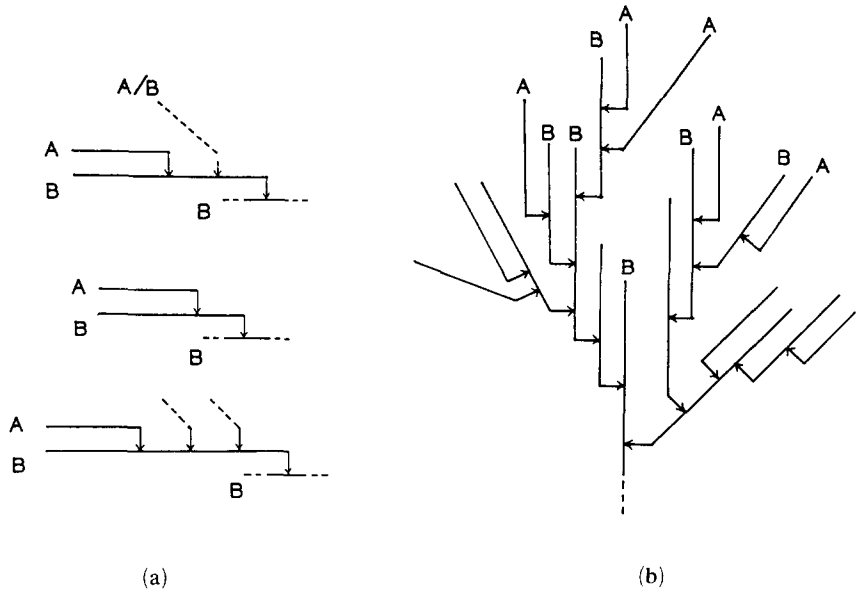

Fig. 13. (a) Possible structures of A- and B-chains, showing B-chains with two, one and three interior chains, respectively. (b) Segment of glycogen macromolecule showing the consequences of different lengths of B-chains, and with some B-chains carrying one or more A-chains.

shortest possible B-chain with a single exterior and interior chain could contain about 13 glucose residues. Other B-chains consisting of a single exterior and many interior chains could comprise 50 or more glucose residues, but the proportion of such chains is very small, since the average length of *all* the B-chains is 18, and the smallest cannot be much less than 13. Figure 13 shows a B-chain with three interior chains, which amounts to about 23 glucose residues. The consequences of the range of sizes of the B-chains is shown in Fig. 13(b) which represents only a segment of a glycogen macromolecule. Some of the B-chains are shown to be carrying more than one A-chain; the proportion of these will be related to the overall A:B-chain ratio. If this ratio was 0·7:1, the actual numerical values for the CL of the A- and B-chains would differ slightly from those given above, but the overall concepts would be unchanged.

The distribution of CL (chain profile) as examined by complete debranching, followed by gel filtration (see p. 50) has been investigated in several laboratories, following the first report by Gunja-Smith *et al.* (1970*b*). Perhaps the best illustration of a chain profile is shown in Fig. 14, from the work of Gidley and Bulpin (1987) on a mussel (*Mytilus edulis*) glycogen, where ^{1}H-NMR analysis has identified the various

chain lengths. The smallest chains with DP >6 represent the A-chains, and this value may be related to the specificity of the branching enzyme, which in mammalian tissues readily transfers chains of six or seven glucose residues. However, the chain profile may also be related to the nutritional state of the organism at the time of isolation of the glycogen. In mammalian tissues, fasting will reduce the glycogen content, and under conditions in which the content of amylo-(1→6)-glucosidase is rate limiting, the exterior chain length could be reduced by phosphorolysis.

The interior of a glycogen molecule must consist mainly of B-chains, although the presence of some 'buried' A-chains, as suggested by French (1964) remains a distinct possibility. This interior structure has been examined by Palmer *et al.* (1983*b*) who studied the ordered and sequential exo-hydrolysis of the inter-chain linkages in oyster glycogen using *Cytophaga* isoamylase. At 34% debranching, the maltosaccharide products had DP 6·7 and none had a DP >16·5, whereas at 61% debranching, the DP of the maltosaccharide products was 9·8, and included products with DP 49. These and other results showed that the

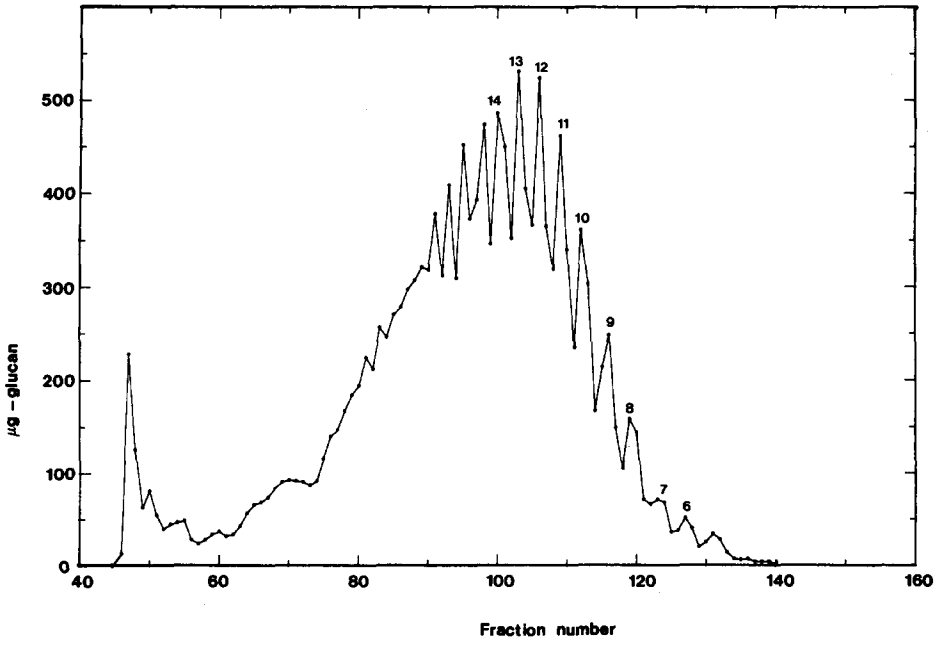

Fig. 14. Chromatography on Bio-Gel P-4 of debranched mussel glycogen. The chain lengths of resolved oligomers (as found by ^{1}H-NMR analysis) are given above the relevant peaks. From Gidley and Bulpin (1987), reproduced by permission of Elsevier, Amsterdam.

DP of the maltosaccharide products released by isoamylolysis increased as a direct function of the degree of debranching, i.e. the length of the constituent chains increased as a function of their depth within the macromolecule. This finding was compatible with a spherical Meyer-type structure.

The compact nature of the interior of the molecule was also shown by Misaki and Yano (1985) (see Fig. 10), where the successive digestions with pullulanase show that many of the branch points are separated by only two, three or four glucose residues. Superimposed on this pattern of branching are the very highly branched regions which are resistant to α-amylase and give rise to the α-macrodextrins (see p. 63). These overall results clearly establish that glycogen does not have a regularly branched structure.

As previously mentioned, the illustration of a macromolecule containing literally thousands of chains is very difficult. The best representation that has been seen is shown in Fig. 15, and has been kindly provided by Professor T. Yamamoto. It is based on his enzymic degradation studies of shellfish glycogen β-limit dextrin (Yamamoto & Limeki, 1975).

α- AND β-PARTICLES OF GLYCOGEN

When glycogen was extracted from tissues by mild procedures at 0–4°C, it was isolated as very large particles with molecular weights $\sim 10^9$, many of which showed characteristic rosette patterns when examined by electron microscopy. The tissues examined included *Ascaris lumbricoides* and rabbit liver (Orrell & Bueding, 1958), liver flukes (*Fasciola hepatica*; Bueding & Orrell, 1961), and rat liver (Drochmans, 1962). The above and related work has been reviewed by Orrell *et al.* (1964). In general, the molecular weight of the cold-water extracted glycogen was at least ten times greater than glycogen extracted with cold TCA, and from 50 to 100 times that of alkali extracted glycogen (Orrell & Bueding, 1958).

The large α-particles had a diameter ranging from 60 to 200 nm and were not affected by reagents which were known to rupture peptide or hydrogen bonds such as 8 M urea, 8 M guanidine, 2 M thiocyanate, 8 M lithium bromide and 1% of various detergents (Orrell *et al.*, 1964). Prolonged incubation with trypsin, chymotrypsin, and bacterial proteases had no effect on the sedimentation characteristics of cold-water extracted glycogen. Protein-bonded aggregation was therefore ruled out.

However, the concept that glycogen exists as β-particles of molecular weight $\sim 10^7$, or as the very large α-particle, is perhaps too simple to account for all the observations in the literature. There is, in fact,

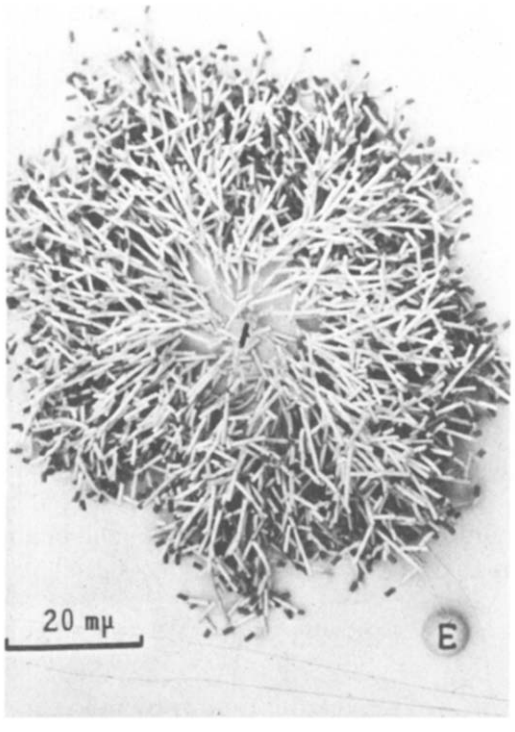

Fig. 15. Model of shellfish glycogen β-limit dextrin. From Yamamoto and Limeki (1975), reproduced by permission of the Japanese Society of Starch Science.

evidence for two sizes of β-particles. Drochmans and Dantan (1968) reported that the β-particles released from α-particles by treatment at pH 3·0 were larger than the β-particles measured inside the untreated α-particles. The average diameters were 48 nm and 38 nm respectively, corresponding to molecular weights of about 20×10^6 and $10-15 \times 10^6$. Size distribution curves showed that both series of β-particles were not homogeneous populations, but two families of particles with different degrees of agglomeration of subunits. The smallest subunits were about the same size as glycogen extracted from tissues with hot alkali having a molecular weight of $1-5 \times 10^6$. In apparent agreement with these suggestions, Geddes *et al.* (1977) have proposed that the fundamental glycogen–protein unit was a single protein chain to which two or three glycogen β-particles were covalently attached (see Fig. 16(a)). This unit would have a molecular weight in the range $20-40 \times 10^6$. The very large α-particles could contain up to about 50 of these fundamental units held together by disulphide bridges. As described previously (p. 57), the

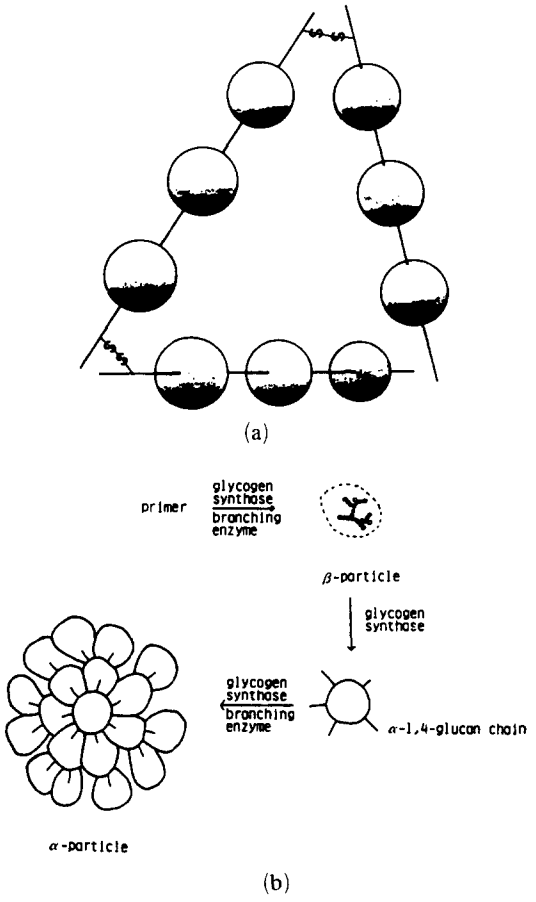

Fig. 16. (a) A schematic representation of the mode by which high molecular weight glycogen is constructed. The balls represent glycogen β-particles and these are built around protein (solid black line). The 'fundamental units' of three β-particles on protein backbones form large conglomerates by disulphide (—S—S—) bridging. From Geddes *et al.* (1977). (b) Proposed model of glycogen α-particle by Hata *et al.* (1984). Reproduced by permission of the Japanese Society of Starch Science.

evidence for disulphide bridges was based on the reaction of 'glycogen' with 2-mercaptoethanol and iodoacetamide. The detailed experimental conditions for the reduction of possible disulphide bridges are critical (Crestfield *et al.*, 1963). Hydroiodic acid (which could presumably dissociate α-particles) and iodine are by-products, and the reduction should be carried out in the absence of oxygen and with the exclusion of light in working up the solution. There is no reference to any of these precautions in the reports by Geddes and his coworkers. Moreover, the

glycogenin molecule is now known to contain only two cysteine residues (Campbell & Cohen, 1989).

An alternative theory for the aggregation of β-particles was discussed by Hata *et al.* (1984). Using electron microscopy to obtain size frequency histograms, they showed that the α-particles were stable to 0·1 M and 0·5 M sodium hydroxide at room temperature over 42 h, 2-mercaptoethanol followed by iodoacetamide, chymotrypsin and alkaline protease, but were degraded by a 30-s treatment with salivary or *Bacillus subtilis* α-amylase. This treatment gave products of the same size as β-particles, i.e. 20–30 nm in diameter. This result supported the hypothesis of Nakamura (1977) that the β-particles were linked together by a small number of elongated exterior chains to give the rosette α-particle (see Fig. 16(b)). This hypothesis recalls other experiments by Drochmans (1973) in which β-particles were incubated with glucose 1-phosphate and phosphorylase. Electron microscopy showed that a limited number of long polysaccharide chains were formed which emanated from the β-particles. However, this theory is not in accord with the dissociation of α-particles at pH 3·0 described by Drochmans (1962).

A third possibility, which was discussed by Whelan and Cameron (1964), concerns the role of inorganic ions in the aggregation of β-particles. The presence of phosphate groups in glycogen has already been noted (p. 56). Their involvement in aggregation would require their location on the surface of the molecule. The relative amount of phosphate required to form an effective bond between adjacent β-particles is not known, nor is the distribution of phosphate groups in glycogens from invertebrate and other non-mammalian sources.

The nature of the interconversion of α- and β-particles was discussed in detail at the CIBA Symposium (Whelan & Cameron, 1964). In 1990, this problem remains an enigma, which still requires a convincing solution. It is tantalising to realise that the answer lies in the rat liver glycogen synthase preparations used *in vitro* by Parodi *et al.* (1969) who synthesised particulate glycogen from UDPG. However, there is a further complication in that in liver, the α-particles are present only in the lysosomes, where they amount to about 10% of the glycogen, with the remainder in the cytosol as β-particles. Liver glycogen is therefore structurally and metabolically heterogeneous (Geddes, 1985).

MINOR COMPONENTS OF GLYCOGEN

Although glycogen has so far been regarded as a homopolymer of D-glucose, the literature contains several reports of the presence of small

proportions of other monosaccharide residues, and more recently, of phosphate ester groups.

The possible presence of D-fructose as a component of rabbit liver glycogen followed from the isolation of about 5% of maltulose after α-amylolysis (Peat *et al.*, 1952*a*). Even smaller amounts were present in α-amylolysates of waxy maize starch. The origin of the fructose was the subject of continuing investigations (Roberts & Whelan, 1960) but no further details appear to have been reported. Attempts in this laboratory to confirm this observation gave inconclusive results.

Some years later, Nordin and Hansen (1963) identified D-galactose in acid hydrolysates of liver glycogen from chicks fed toxic amounts of D-galactose. The galactose to glucose ratio was about 1:500. This galactose may have resulted from a very limited and inefficient transfer involving glycogen synthase, although in general, galactose, galactose 1-phosphate and UDP-D-galactose are inhibitors of the corresponding glucose-metabolising enzymes.

D-Glucosamine has been found in some liver glycogens. When D-galactosamine was injected intraperitoneally into rats, the liver glycogen could contain up to 10% of glucosamine (Romero *et al.*, 1980). D-Galactosamine was converted into UDP-D-galactosamine and then epimerised to UDP-D-glucosamine; the amino-sugar was then incorporated into glycogen by glycogen synthase. This glucosamine could be released from glycogen by phosphorylase giving glucosamine 1-phosphate, and also reincorporated into glycogen by the same enzyme. Further enzymic analyses with glucoamylase showed that the amino-sugar was distributed randomly in the macromolecule. Moreover, D-glucosamine-containing glycogen was a substrate for isoamylase, glycogen synthase and branching enzyme.

In a later study, Kirkman and Whelan (1986) reported that D-glucosamine occurred endogenously in both rabbit and pig liver glycogen to the extent of about 1 nmol per 10 mg glycogen. This glucosamine could be released by α-amylolysis, while on β-amylolysis, about 50% was released, indicating a random distribution within the molecule. On average, 1–2 molecular proportions of glucosamine were present per molecular weight of 10^7; its presence appears to be adventitious, and to be confined to liver and kidney glycogens.

In a review of the classical literature on the chemistry of glycogen, Meyer (1943) concluded that glycogen was not united to protein or phosphoric acid by primary valences in its native condition. It has already been seen how protein is an integral part of glycogen structure. The presence of phosphate in glycogen must now be considered.

Fontana (1980) administered radioactive phosphate to rats and a mould (*Neurospora crassa*) and the liver and mould glycogens were

isolated, fractionated and degraded. The ^{32}P and glycogen could not be separated, and α-amylolysis gave, as major products, maltotriose and maltotetraose phosphorylated at C6 of one glucose residue. On β-amylolysis, all the ^{32}P remained in the β-limit dextrin. However, acid and alkaline hydrolysis experiments showed the presence of two different types of glycogen bound ^{32}P-phosphate. In later experiments with rabbit muscle and liver glycogen (Rodriguez *et al.*, 1986), similar evidence for two types of phosphate ester group in glycogen was obtained. Rabbit muscle glycogen contained one phosphate group per 600 glucose residues, 30% of which was present as glucose 6-phosphate, and 70% as a phospho-diester, in which the C3 hydroxyl could be involved. Rabbit liver glycogen contained only about one-tenth of the phosphate as muscle glycogen, but again, 30% was present as glucose 6-phosphate. However, the liver glycogen was heterogeneous with respect to phosphate content and could be fractionated to give glycogens containing between 2 and 150 nmoles phosphate/10 mg glycogen. More recently, Lomako *et al.* (1989) have shown that muscle glycogen also shows heterogeneity, with fractions containing one phosphate per 1900 glucose residues increasing to one phosphate per 200 glucose residues. The degree of conversion into glucose by glucoamylase was 74 and 48% respectively.

The mode of incorporation and function of the two types of phosphate group are under investigation. Fontana (1980) noted the possible contribution of phosphate in the maintenance of the particulate structure of glycogen. Whether phosphate is present in glycogens from invertebrate, bacterial and protozoal tissues remains to be determined.

GLYCOGEN STORAGE DISEASES

The glycogen storage diseases are a group of human diseases, fortunately rare, which are characterised by the accumulation of abnormal amounts of glycogen in different tissues, especially in the liver. Normal liver contains up to 5% of glycogen, but glycogen contents well in excess of 10% have been recorded, whilst in other cases, muscle tissue which normally contains up to 1% of glycogen, had glycogen contents ranging from 4 to 11%. This accumulation is due to the relative absence of a glycogen-metabolising enzyme. Although in most cases the deposited glycogen has a normal structure, in some cases, glycogen with an abnormal structure has been found. The disease is accompanied by various clinical manifestations, e.g. hepatomegaly, hypoglycaemia, but detailed discussion of these is outside the scope of this review. Nevertheless, as clinical and biochemical information began to accumulate, it

TABLE 3
Classification of the Glycogen Storage Diseases[a]

Type	*Disease*	*Organ affected*	*Glycogen structure*	*Enzyme deficiency*
I	Von Gierke	Liver, kidney	Normal	Glucose 6-phosphatase
II	Pompe	Generalised	Normal	Lysosomal α-glucosidase
III	Limit dextrinosis	Liver and/or muscle	ϕ-Dextrin	Amylo-(1 → 6)-glucosidase
IV	Andersen	Liver	'Amylopectin type'	Branching enzyme
V	McArdle	Muscle	Normal	Phosphorylase
VI	Hers	Liver	Normal	Phosphorylase kinase
VII	Tarui	Muscle	Normal	Phosphofructokinase

[a]Various sub-types may exist (Van Hoof & Hers, 1967; Huijing, 1970), and Types VIII and IX, related to Type VI, have been described in some reviews.

became apparent that different types of the disease existed, and the present classification of these is given in Table 3.

The biochemical studies on the glycogen storage diseases began in the 1950s in the laboratory of Carl and Gerty Cori, who pioneered many of the techniques used for the enzymic analysis of glycogen structure (see Illingworth & Cori, 1952), and the assay of tissue samples for glycogen-metabolising enzymes. Notable contributions were made in the following decade by Hers and his coworkers, particularly with respect to Types II and VI diseases. The first enzymic deficiency reported was the absence of glucose 6-phosphatase from cases of Type I disease (Cori & Cori, 1952). This enzyme occurs only in the liver and kidney, and muscle glycogen is unaffected.

The biochemical studies on glycogen storage diseases led not only to the classification shown in Table 3, but also provided fundamental information on the in vivo significance of certain glycogen-metabolising enzymes. For example, the work of Hers on Type II disease, due to the absence of the lysosomal α-glucosidase (acid maltase), established the importance of the non-phosphorolytic pathway for the degradation of glycogen in the lysosomes (Hers, 1963). The existence of Type V McArdles disease showing glycogen *synthesis* in the absence of muscle phosphorylase, clearly demonstrated the *degradative* role of this enzyme, rather than its involvement with glycogen synthesis. In Type III disease, the deficiency in amylo-(1 → 6)-glucosidase led to the accumulation of ϕ-dextrin, whilst in type IV disease, the relative lack of branching enzyme gave an amylopectin-type polysaccharide with only 4% of branch points instead of the normal 8%. However, the origin of the 4% remains a mystery, if branching enzyme activity cannot be demonstrated in tissue samples.

The existence of the glycogen storage diseases also stimulated the development of enzymic methods for the structural analysis of glycogen on the milligram scale (see Kjolberg & Manners, 1962). The literature on these diseases is now very extensive, but the reviews by Brown and Brown (1968) and by Hers and Van Hoof (1968) are authoritative accounts of the development of the more biochemical aspects.

GLYCOGENS FROM PLANT, FUNGAL AND OTHER SOURCES

Although glycogen is recognised as the carbohydrate reserve of animal tissues, glycogen-type polymers have been isolated from several higher plants, algae, bacteria, fungi and protozoa. The extent of the characterisation of these polymers has varied considerably. The first biological

TABLE 4
Properties of Some Glycogens from Plants, Fungi and Other Tissues

Sample	*CL*	*α-Amylolysis limit (%)*	*β-Amylolysis limit (%)*	*ECL*	*ICL*	*Reference*
(A) Higher plants						
Cecropia peltata	13	85	53	9	3	Marshall and Rickson (1973)
Maize phytoglycogen	10–16	—	45–60	6–11	3–4	Boyer and Liu (1983)
Sorghum phytoglycogen	12–16	—	49–65	8–11	3–4	Boyer and Liu (1983)
(B) Fungi						
Candida albicans	16	81	45	9	6	Yamaguchi *et al.* (1974)
Corticium rolfsii	8	36	32	5	2	Hiura *et al.* (1982)
Neurospora crassa	10	58	34	6	3	Hiura *et al.* (1982)
Polyporus circinatus	12	52	23	5	6	Fontana and Zancan (1977)
Saccharomyces cerevisiae (brewers' yeast)	13	68	44	8	4	Manners (1971)
(C) Algae and protozoa						
Prototheca zopfii	14	77	49	9	4	Manners *et al.* (1973)
Tetrahymena pyriformis	13		44	8	4	Manners and Ryley (1952)
Trichomonas foetus	15	63	60	11	3	Manners and Ryley (1955)

studies depended on iodine-staining techniques of D-glucans which could be hydrolysed by amylases, but were inadequate for quantitative characterisation. In later studies, CL values were reported by various methods; in some examples, β-amylolysis data were described which enabled ECL and ICL to be calculated. More recently, chain profiles for several polymers have been reported (for example, Weber & Wober, 1975; Boyer & Liu, 1983). Some of the curves obtained were not as smooth as for rabbit liver glycogen; whether the profiles were structurally significant, or were artefactual due to variations in gel filtration materials or to the mode of presentation to the results (see p. 50) is not yet clear. Examples of CL determination include values of 9 for the alga *Anacystis nidulans*, 14 for *Escherichia coli*, 8 for *Arthtrobacter* sp. (Weber & Wober, 1975), 9–16 for *Blastocladiella emersonii* (Norrman *et al.*, 1975) and 12 for sugar cane phytoglycogen (Roberts *et al.*, 1988). The results of more complete structural analyses are summarised in Table 4.

The presence of 'glycogen' in so many plant, bacterial, fungal and protozoal cells is of great interest from a comparative biochemical point of view. Future research will no doubt establish whether a glycogenin-like protein and phosphate ester groups are structural components. A preliminary survey (Singh *et al.*, 1988) has already shown that a glucose-acceptor protein (glycogenin) was present in sweet potato, banana and plantain. Whether this is related to phytoglycogen synthesis or to starch synthesis remains to be determined.

It is not possible to discuss the details of the structure and metabolism of these glycogen-type polymers in this review, but many aspects of yeast, fungal and bacterial glycogens have been considered elsewhere (Manners, 1971; Manners & Sturgeon, 1982; Preiss & Walsh, 1981).

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