THE ENZYMIC DEGRADATION OF POLYSACCHARIDES *

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Introduction

THE main structural features of a polysaccharide can usually be determined by the chemical methods of methylation and periodate oxidation.[1] Certain limitations in these methods have, however, become apparent ; it is, for example, difficult to effect complete methylation of a highly branched polysaccharide,[2] or to avoid " over-oxidation " of the reducing group of a polysaccharide during periodate oxidation.[3] Additional methods of structural analysis have therefore been sought. One possible method is a study of the degradation of the polysaccharide by purified *hydrolytic* enzymes. This Review will outline recent progress in the chemistry of such enzymes (polysaccharases), with particular reference to their activity *in vitro*, since in many instances this has yielded new information on the fine structure of a polysaccharide. Physiological aspects of polysaccharase action will not be discussed here.

Before reviewing the various classes of polysaccharases, the methods of polysaccharase chemistry will be considered. Enzymic action involves the hydrolysis of a glycosidic linkage ; in Fig. 1 the scission of a β-1 : 4-manno-

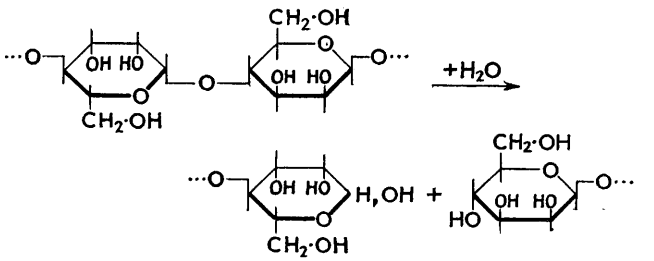


FIG. 1

Hydrolysis of a β-1 : 4-mannosidic linkage.

sidic linkage is shown. The course of hydrolysis of a soluble polysaccharide can be followed by means of (*a*) the decrease in viscosity or turbidity of the polysaccharide, (*b*) the change in optical rotation, (*c*) the increase in reducing power, (*d*) chromatography of the polysaccharide–enzyme mixture.

[1] See, for example, Percival, " Structural Carbohydrate Chemistry ", Garnet Miller, London, 1953, 2nd edn. ; Pigman and Goepp, " Chemistry of the Carbohydrates ", Academic Press, New York, 1948 ; Whistler and Smart, " Polysaccharide Chemistry ", Academic Press, New York, 1953.

[2] Cf. Bell, *Ann. Reviews Biochem.*, 1949, **18**, 87 ; Bell and Manners, *J.*, 1954, 1891.

[3] Cf. Head and Hughes, *J.*, 1954, 603.

* The Enzymic Synthesis of Polysaccharides has been reviewed recently by Barker and Bourne (*Quart. Reviews*, 1953, **7**, 56).

Paper chromatography has been widely used for preliminary identification of the products of polysaccharase action. The degradation of an insoluble polysaccharide, *e.g.*, cellulose, can be examined by methods (*c*) and (*d*). Ideally, the mode of action (action pattern) of a polysaccharase is determined by investigating the degradation of a polysaccharide of known structure; the enzyme may then be used in structural investigations of related polysaccharides. For example, the action pattern of salivary α-amylase [4] and of the cereal β-amylases [5] has been determined by using amylose and amylopectin (the linear and branched components of starch) as substrates; with this knowledge, the degradation of various bacterial and protozoal α-1 : 4-glucosans by these enzymes has given structural data which confirm and extend those obtained by chemical methods. Unfortunately, present knowledge of the action pattern of many polysaccharases is far from complete; accordingly reference to the enzymic degradation of mucopolysaccharides, plant gums and mucilages, and many other heteropolysaccharides will be omitted from this Review. In the following sections certain aspects of enzymic structural analysis will be discussed; a general account of several classes of polysaccharases then follows.

Determination of the Component Monosaccharides in a Polysaccharide.—The complete acid hydrolysis of a polysaccharide is usually a satisfactory method for determining the component monosaccharides. In a number of instances, enzymic experiments have been of value, *e.g.*, the presence of L-galactose in an enzymic hydrolysate of snail galactogen has confirmed the presence of both isomers of galactose in this polysaccharide.[6] In general, enzymic hydrolysis is more specific than acidic hydrolysis; the presence of D-glucose in acid hydrolysates of inulin does not exclude the possibility that the glucose has arisen from a contaminating glucosan; however, the liberation of glucose from inulin by inulase provides evidence in favour of a glucofructosan structure for inulin.[7]

Determination of the Linkages in a Linear Polysaccharide.—Identification of the end-products of polysaccharase action will usually enable the configuration and the carbon atoms involved in the inter-residue linkage to be determined. Several examples are given in Table 1.

The end-products will depend on the action pattern of the polysaccharase. An enzyme catalysing the *stepwise* hydrolysis of *every* glycosidic linkage will produce only the constituent monosaccharide(s): *e.g.*, glucose is the

[4] Whelan and Roberts, *Nature*, 1952, **170**, 748; *J.*, 1953, 1298.

[5] See Manners, *Ann. Reports*, 1953, **50**, 288; Myrbäck and Neumüller, in "The Enzymes", by Sumner and Myrbäck, Academic Press, New York, 1950, Vol. I, Part 1, p. 653.

[6] Weinland, *Biochem. Z.*, 1953, **324**, 74.

[7] Dedonder, *Bull. Soc. Chim. biol.*, 1952, **34**, 157.

[8] Tracey, *Biochem. Soc. Symp.*, 1953, **11**, 49.

[9] Jeanes, Wilham, Jones, Tsuchiya, and Rist, *J. Amer. Chem. Soc.*, 1953, **75**, 5911.

[10] Duncan, Manners, and Ross, *Biochem. J.*, 1954, **57**, xviii, and unpublished work.

[11] McCready and McComb, *Agric. Food Chem.*, 1953, **1**, 1165.

[12] Jones and Reid, *J.*, 1954, 1361; Altermatt and Deuel, *Helv. Chim. Acta*, 1954, **37**, 770.

TABLE 1. *Products of polysaccharase action*

Polysaccharide	Enzyme	Major product(s)	Main repeating linkage	Ref.
Cellulose .	Cellulase	Cellobiose	β-1 : 4-Glucosidic	8
Dextran .	Dextranase	*iso*Maltose *iso*Maltotriose	α-1 : 6-Glucosidic	9
Laminarin .	β-Glucosanase	Glucose Laminaribiose Laminaritriose	β-1 : 3-Glucosidic	10
Pectic acid .	Polygalacturonase	Galacturonic acid Digalacturonic acid Trigalacturonic acid	α-1 : 4-Galacturonidic	11, 12
Starch . .	α-Amylase	Maltose	α-1 : 4-Glucosidic	5
Xylan . .	Xylanase	Xylose Xylobiose Xylotriose	β-1 : 4-Xylosidic	10

sole product of " amyloglucosidase " (a polysaccharase from *Aspergillus niger*) action on amylose.[13] If, however, a polysaccharase catalyses a *stepwise* hydrolysis of *alternate* glycosidic linkages, the sole end-product will be a disaccharide containing the same glycosidic linkage as the polysaccharide. Thus, certain samples of amylose are quantitatively converted into maltose by β-amylase ; [14] the inter-residue linkage in amylose is therefore of the α-1 : 4-glucosidic type. The most common type of polysaccharase action involves *random* hydrolysis of the substrate thereby producing a series of oligosaccharides : *e.g.*, pectic acid (a polymer of galacturonic acid) is degraded by a purified fungal polygalacturonase to give a mixture of mono-, di-, tri-, and tetra-galacturonic acids.[11] Random and stepwise hydrolysis may be distinguished by means of viscometric and reductometric measurements ; in the former action, the viscosity of the substrate falls sharply with concomitant production of only a few reducing groups, whereas in stepwise hydrolysis, reducing groups are quickly liberated, and the viscosity decreases slowly and regularly.

Determination of the Linkages in a Branched Polysaccharide.—Branched polysaccharides contain more than one type of glycosidic linkage : the majority of these are " inter-residue " linkages which unite the monosaccharides in the " unit-chains " ; * the remainder are " inter-chain "

[13] Kerr, Cleveland, and Katzbeck, *J. Amer. Chem. Soc.*, 1951, **73**, 3916.

[14] Meyer, *Experientia*, 1952, **8**, 405.

* Chemical analysis of a branched polysaccharide of high molecular weight may reveal that, on a statistical basis, the ratio of non-terminal to terminal monosaccharide residues is, for example, 15 : 1. The polysaccharide is thus composed of a large number of repeating units of 15 monosaccharide residues. For convenience, these units may be referred to as " unit-chains " ; it must be noted, however, that figures for unit-chain lengths (which are given to the nearest whole number) represent mean values, and that individual unit-chains vary considerably in length.

linkages. Thus amylopectin contains *ca.* 4% of α-1 : 6-linkages which inter-link the constituent unit-chains, each of which comprises *ca.* 20 α-1 : 4-linked glucose residues.[5] Random enzymic hydrolysis will therefore produce a mixture of oligosaccharides belonging to more than one homologous series. The majority of these will contain inter-residue linkages; the remainder will consist either of a disaccharide containing the inter-chain linkage, or higher saccharides containing this linkage and one or more inter-residue linkages, depending on the specificity of the polysaccharase. Salivary α-amylase, for example, cannot hydrolyse α-1 : 4-linkages which are adjacent to 1 : 6-linkages in amylopectin: accordingly, the products of salivary amylolysis include a series of branched oligosaccharides (α-dextrins) which contain a 1 : 6-linkage and three or more α-1 : 4-linkages.[4]

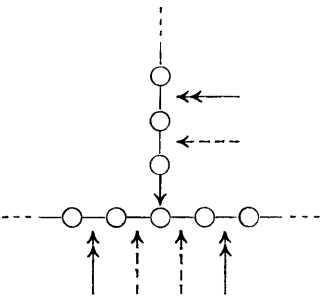


FIG. 2

α-Amylolysis of branched chain in amylopectin.

○ Glucose residue. — α-1 : 4-Linkage. ⟶ α-1 : 6-Linkage.
⟶ Linkage susceptible only to *A. oryzæ* α-amylase.
⟶ Linkage susceptible to salivary and to *A. oryzæ* α-amylase.

In contrast, *Aspergillus oryzæ* α-amylase can hydrolyse α-1 : 4-linkages adjacent to a 1 : 6-linkage,[15] and the end-products of amylolysis therefore include *iso*maltose (see Fig. 2). These types of enzymic structural analysis have been greatly facilitated by the development of chromatographic methods for the separation of oligosaccharides.[16]

Determination of the Degree and Type of Branching in a Polysaccharide.—This type of investigation requires a detailed knowledge of the action pattern of highly purified polysaccharases, and is, at present, limited to starch-type polysaccharides since the requisite knowledge for polysaccharases other than amylases is not yet available. Details of the methods developed for the determination of the degree of branching (*i.e.*, end-group assay) and type of branching (*i.e.*, single or multiple) in amylopectins will be described on p. 84—87.

Criteria of Purity and Activity of Polysaccharases.—For the structural analysis of polysaccharides by enzymic methods, highly purified enzyme

[15] Montgomery, Weakley, and Hilbert, *J. Amer. Chem. Soc.*, 1949, **71**, 1682.
[16] *E.g.*, see Whelan, Bailey, and Roberts, *J.*, 1953, 1293; Derungs and Deuel, *Helv. Chim. Acta*, 1954, **37**, 657.

preparations are essential. Recent progress in the development of fractionation procedures for proteins (for reviews see ref. 17) has enabled polysaccharases of a high degree of purity to be prepared. In addition, if the conclusions drawn from enzymic studies are to be valid, the *enzymic homogeneity* of the polysaccharase must be ensured; other carbohydrases or transglycosylases are often associated with polysaccharases. The presence of cellobiase in cellulase or of maltase in amylase preparations led early workers to conclude that glucose was the primary product of "cellulase" or "amylase" action on cellulose or starch respectively. The possibility of transglycosylase impurities has only recently been realised.[18] Transglycosylases catalyse the transfer of a glycosyl unit from a donor to a suitable acceptor: *e.g.*, *Aspergillus niger* extracts convert maltose into a mixture of glucose and panose (4-α-*iso*maltosyl-D-glucose) [19] (cf. Fig. 3).

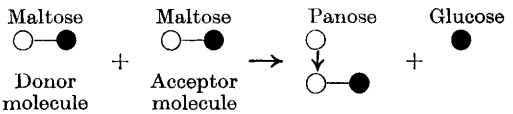


FIG. 3

Transglucosylation of maltose by A. niger *extracts.*

Symbols as for Fig. 2. ● Reducing glucose molecule.

Such amylolytic extracts cannot be used, therefore, in experiments aimed at the characterisation of the inter-chain linkages in starch-type polysaccharides.

Artefacts may arise during polysaccharase action in two additional ways. First, some polysaccharases, under certain conditions, show *synthetic* activity. Thus, emulsin when incubated with high concentrations of glucose, catalyses the synthesis of gentiobiose and other β-linked disaccharides.[20] The same enzyme hydrolyses certain β-glucosans, *e.g.*, laminarin and yeast glucan; [10] the presence of disaccharides in these hydrolysates cannot be taken as unambiguous evidence for the nature of the inter-residue and inter-chain linkages since the possibility of "reversion" from glucose remains. All polysaccharases must therefore be examined for synthetic activity. Secondly, if a mixture of two purified enzymes is used in a structural analysis, a "synergic" reaction may occur. In such reactions, the specificity of one enzyme is altered by the presence of the second, and abnormal hydrolysis occurs. Two examples of a synergic reaction with α-amylase have been discovered recently [21, 22] and are discussed on p. 87;

[17] Schwimmer and Pardee, *Adv. Enzymology*, 1953, **14**, 375.

[18] *E.g.*, the transglucosylase (transglucosidase) activity of various amylase preparations had been determined by Pan, Nicholson, and Kolachov, *Arch. Biochem. Biophys.*, 1953, **42**, 421.

[19] *Idem, J. Amer. Chem. Soc.*, 1951, **73**, 2547; Wolfrom, Thompson, and Galkowski, *ibid.*, p. 4093.

[20] Peat, Whelan, and Hinson, *Nature*, 1952, **170**, 1056.

[21] Whelan, *Biochem. Soc. Symp.*, 1953, **11**, 17.

[22] Schwimmer and Garibaldi, *Cereal Chem.*, 1952, **29**, 108.

the possibility of similar reactions with other polysaccharases must not be overlooked.

Enzymic Degradation of Starch

Starch, the reserve carbohydrate of many plants, contains two distinct polysaccharides—amylose and amylopectin. The amylose content of starch is usually *ca.* 20%, an amylose molecule consisting essentially of a linear chain of several thousand glucose residues united by α-1 : 4-linkages. Amylopectin is a highly branched molecule, composed of several hundred unit-chains, each of which comprises 20—25 α-1 : 4-linked glucose residues; the unit-chains are inter-linked by glucosidic linkages from the reducing group to $C_{(6)}$ of a glucose residue in an adjacent chain.[23]

Four main groups of *hydrolytic* enzymes attack starch : (*a*) α-amylases, which catalyse a random hydrolysis of α-1 : 4-linkages; (*b*) β-amylases, which catalyse a stepwise hydrolysis of alternate α-1 : 4-linkages; (*c*) "glucose-producing" amylases, which catalyse a stepwise hydrolysis of all α-1 : 4-linkages; and (*d*) "debranching" enzymes, which hydrolyse α-1 : 6-linkages.

Starch is also degraded by *Bacillus macerans* amylase (Schardinger dextrinogenase); this enzyme, by a transference action, converts starch into a mixture of cyclic dextrins.[5]

α-Amylases.—α-Amylases have been isolated in purified form from many sources, *e.g.*, barley malt, mammalian pancreatic and salivary secretions, and several bacterial and fungal extracts.[5, 14] Several α-amylases have been crystallised.[24] Superficially, all α-amylases catalyse a similar reaction, *viz.*, random hydrolysis of α-1 : 4-linkages, shown initially by a rapid decrease in the viscosity, turbidity, and iodine-staining power of the substrate and, later, by the production of oligosaccharides. The initial enzyme action (dextrinisation) involves degradation of the substrate into α-dextrins which contain 6—10 glucose residues. α-Dextrins from amylose are linear molecules, whereas those from amylopectin have branched structures, since α-amylases cannot hydrolyse α-1 : 6-linkages. In the later stages of α-amylolysis (the saccharification stage), α-dextrins are further broken down to reducing sugars. Recent investigations have revealed important differences in the mode of action of different α-amylases. During the α-amylolysis of amylose, the achroic stage of hydrolysis is reached when 23% of the linkages have been hydrolysed by swine pancreatic α-amylase, whereas with human salivary or *Aspergillus oryzæ* amylase, only 15 or 12% of the linkages are broken at this stage.[25] These differences reflect some variation in dextrinisation action. Specificity differences during saccharification have also been demonstrated. Malt α-amylase and *B. subtilis* amylase, unlike salivary amylase, can hydrolyse the linkage adjacent to

[23] For reviews of starch chemistry see Bourne, *Chem. and Ind.*, 1951, 1047; Meyer and Gibbons, *Adv. Enzymology*, 1951, **12**, 341; Hassid in "Organic Chemistry", Vol. IV, Ed. by Gilman, Wiley, New York, 1953, p. 901.

[24] Meyer, *Angew. Chem.*, 1951, **63**, 153.

[25] Tung Kung, Hanrahan, and Caldwell, *J. Amer. Chem. Soc.*, 1953, **75**, 5548.

a reducing group, thereby liberating glucose ; [26] they can also hydrolyse maltotriose.[4, 26] Since the composition of the end-products of α-amylolysis is dependent on the enzyme source, further discussion will be limited to salivary amylolysis, since the action pattern has been most clearly defined, by Whelan and Roberts.[4]

Potato amylose, on complete salivary amylolysis, gave an apparent conversion into maltose (from reducing-power determinations) of 91% ; the end-products[4] were maltose and maltotriose in the molar ratio of 2·39 : 1. Since glucose was not produced, terminal α-1 : 4-linkages must be resistant to enzyme action. If α-amylolysis were completely random, and if all susceptible linkages were hydrolysed at the same rate, the calculated molar ratio of maltose and maltotriose would be 2·35 : 1. Whelan and Roberts therefore postulated that salivary amylolysis of amylose is a random hydrolysis of non-terminal linkages. Experiments using linear maltosaccharides of DP * 4—7 gave maltose and maltotriose in yields which were in agreement with the proposed action pattern. The end-products of salivary amylase action on amylopectin are maltose, maltotriose, and branched α-dextrins ; eight of these dextrins have been isolated, the smallest being a pentasaccharide, and the largest an octasaccharide.[4] These findings

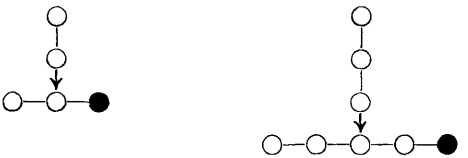


FIG. 4

α-Dextrins from amylopectin.

suggest that the three α-1 : 4-linkages adjacent to a 1 : 6-linkage are resistant to enzyme action. Salivary amylolysis thus involves random hydrolysis of non-terminal α-1 : 4-linkages except those adjacent to an inter-chain linkage.

β-Amylases.—The action pattern of β-amylase, which is known only in certain plants (*e.g.*, wheat, barley, and soya bean) does not appear to depend on the enzyme source. β-Amylolysis consists of stepwise hydrolysis of alternate linkages in a chain of α-1 : 4-linked glucose residues, from the non-reducing end, with the liberation of β-maltose.[5] Enzyme action is arrested by the presence of anomalous linkages in the chain ; these may be inter-chain linkages or ester-phosphate linkages. Unlike α-amylases, β-amylase cannot by-pass such linkages, since interior † chains in branched α-1 : 4-glucosans are not attacked. Linear amylose molecules on β-amylolysis are completely degraded ; other samples (as shown in Table 2) have low β-amylolysis limits and presumably contain a small number of anomalous

[26] Bird and Hopkins, *Biochem. J.*, 1954, **56**, 86.

* DP = Degree of polymerisation.

† Those parts of a unit-chain between two branch-points (cf. Fig. 5).

linkages. β-Amylolysis of amylopectin yields maltose and a β-dextrin of high molecular weight which differs from amylopectin in that the exterior chains (*i.e.*, those parts of a unit-chain between the branch point and the non-reducing terminal group) contain only two or three glucose residues.

Glucose-producing Amylases.—In 1951, the existence of two amylases which yield glucose as the *primary* product of their action on starch was reported. These enzymes catalyse a stepwise hydrolysis of every linkage in a chain of α-1 : 4-linked glucose residues, beginning at the non-reducing terminal linkage. One such amylase, from the mould *Rhizopus delemar*, liberates over 90% of the glucose from amylose, amylopectin, glycogen, and a β-dextrin.[27] This enzyme cannot hydrolyse 1 : 6-linkages, but can by-pass them, thereby attacking interior chains. A second glucose-producing amylase has been isolated from *Aspergillus niger* and named " amyloglucosidase ".[13] The so-called " maltase " from *Clostridium acetobutyricum* also appears to be a glucose-producing amylase since it converts maltose, maltoheptaose, *iso*maltose, and starch almost quantitatively into glucose; it differs from the above mould amylases in that it can hydrolyse both α-1 : 4- and 1 : 6-linkages.[28]

" Debranching " Enzymes.—Important advances have been made recently in studies of enzymes catalysing the hydrolysis of α-1 : 6-inter-chain linkages in starch-type polysaccharides. The debranching enzyme from the potato and broad-bean—R-enzyme—has been purified by Hobson, Whelan, and Peat.[29] R-enzyme hydrolyses the inter-chain linkages in amylopectin, as shown by the increase in the β-amylolysis limits of amylopectin or β-dextrin when incubated with R-enzyme. The 1 : 6-linkages in branched α-dextrins are also hydrolysed by R-enzyme, giving a mixture of linear maltosaccharides.[4] R-enzyme cannot hydrolyse the inter-chain linkages in glycogen—a branched α-1 : 4-glucosan which forms the reserve carbohydrate of animals and differs from amylopectin in that the unit-chains usually comprise only *ca.* 12 glucose residues.

From a brewer's yeast autolysate, a debranching enzyme known as *iso*amylase has been obtained, which hydrolyses the inter-chain linkages in glutinous rice starch producing a more linear polysaccharide of lower molecular weight.[30] *iso*Amylase and R-enzyme are therefore similar in that they hydrolyse *non-terminal* α-1 : 6-linkages.

A second type of debranching enzyme which can only hydrolyse *terminal* α-1 : 6-linkages has been isolated from rabbit muscle, and named amylo-1 : 6-glucosidase; it has no action on amylopectin or glycogen, but can hydrolyse those 1 : 6-linkages which are exposed by the action of muscle phosphorylase on these polysaccharides.[31] Phosphorylases [5, 32] catalyse the degradation of chains of α-1 : 4-linked glucose residues by transferring a

[27] Phillips and Caldwell, *J. Amer. Chem. Soc.*, 1951, **73**, 3559, 3563.
[28] French and Knapp, *J. Biol. Chem.*, 1950, **187**, 463.
[29] Hobson, Whelan, and Peat, *J.*, 1951, 1451; Peat, Whelan, Hobson, and Thomas, *J.*, 1954, 4440.
[30] Maruo and Kobayashi, *Nature*, 1951, **167**, 606.
[31] Cori and Larner, *J. Biol. Chem.*, 1951, **188**, 17.
[32] Bernfeld, *Adv. Enzymology*, 1951, **12**, 379.

glucosyl residue from the chain to inorganic phosphate, according to the equation :

$$[G]_n + m\text{HO.P} \rightleftharpoons m\text{G.1P} + [G]_{n-m}$$

where $[G]_n$ or $[G]_{n-m}$ represents a linear chain of n or $(n - m)$ α-1 : 4-linked glucose residues, HO.P = inorganic phosphate, and G.1P. = α-glucose 1-phosphate. The reaction is reversible. Amylopectin or glycogen contains three types of unit-chain, each of which is linear and composed of α-1 : 4-linked glucose residues, *viz.*, *A-chain*, linked to the molecule only by a 1 : 6-linkage to an adjacent chain ; *B-chain*, to which one or more A-chains are attached, and which is itself linked by a 1 : 6-linkage from the reducing group to an adjacent chain ; *C-chain*, to which other chains are attached and which carries a free reducing group.[33] Muscle phosphorylase shows a different specificity towards glucosidic linkages in the three types of unit-chain.[31] Phosphorolysis of an A-chain is complete except

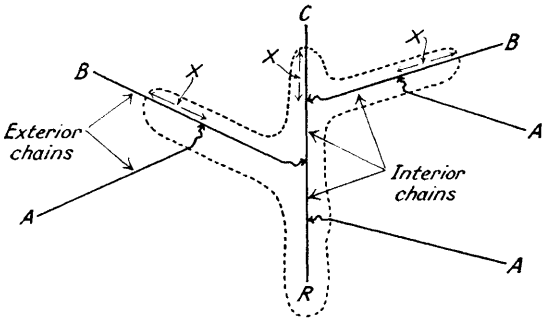


FIG. 5

Phosphorolysis of amylopectin or glycogen.

— Linear chain of α-1 : 4-linked glucose residues.
1 : 6-linkage. A, B, C : Types of unit-chain.
R : Free reducing group. ····· Extent of phosphorolysis.
X : *ca.* 6 glucose residues.

for a single glucose residue which remains attached, by a 1 : 6-linkage, to a B-chain, whereas phosphorolysis of a B- or a C-chain ceases at approximately the sixth residue from the 1 : 6-linkage (see Fig. 5). Amylo-1 : 6-glucosidase action is limited to the hydrolysis of the 1 : 6-linkages which attach single glucose residues to B(or C)-chains in a phosphorylase limit-dextrin of amylopectin or glycogen, thereby yielding glucose.[31]

Structural Analysis of α-1 : 4-Glucosans using Hydrolytic Enzymes

Use of Salivary α-Amylase.—The salivary amylolysis of a glucosan may be used to detect α-1 : 4-glucosidic linkages. Resistance of a glucosan to α-amylase implies that the polysaccharide contains few, if any, sequences of *ca.* 3 or more adjacent α-1 : 4-linkages. Conversely, linear α-1 : 4-glucosans are completely degraded to a mixture of reducing sugars ; thus,

[33] Peat, Whelan, and Thomas, *J.*, 1952, 4546.

potato amylose on α-amylolysis has a Rm value * of *ca.* 91%.[4] The polysaccharide synthesised by the yeast *Cryptococcus neoformans* (*Torula histolytica*), on α-amylolysis, has Rm 93 and therefore has an essentially unbranched structure.[34] Since branched α-1 : 4-glucosans, on α-amylolysis, yield α-dextrins in addition to reducing sugars, the Rm value is correspondingly lower ; † *e.g.*, glycogen [35] (rabbit liver or oyster) has Rm 73. The polysaccharide synthesised by a strain of *Neisseria perflava*, on α-amylolysis, has Rm 72 ; it is therefore a branched α-1 : 4-glucosan.[35] A sample of liver glycogen from a case of von Gierke's disease had Rm 31, indicating that an unusually small proportion of the α-1 : 4-linkages were susceptible to salivary amylase ; [36] further evidence of an abnormal structure was obtained by chemical end-group assay which revealed a chain length of only 6 glucose residues.

Paper-chromatographic examination of an α-amylolytic digest will also provide qualitative evidence of branching in an α-1 : 4-glucosan, since the α-dextrins are easily detected and distinguished from glucose, maltose, and maltotriose. This method has been used to confirm the branched nature of the glucosans synthesised by the protozoa *Trichomonas fœtus* and *T. gallinæ*.[37]

Use of β-Amylase.—β-Amylase has been used (*a*) to detect the presence of anomalous linkages in amyloses, (*b*) to distinguish qualitatively between linear and branched α-1 : 4-glucosans, and (*c*) to determine the exterior chain lengths of branched α-1 : 4-glucosans.

The β-amylolysis limits of several samples of amylose are recorded in Table 2. The low limits are not due to retrogradation of amylose during

TABLE 2. *β-Amylolysis limits of amyloses*

Sample	DP	β-Amylolysis limit *	Ref.
Apple	545	90	39
Maize	—	75	40
Maize	—	68†	38
Maize	490	90	41
Potato	—	88	40
Potato	—	68†	38
Potato	—	76†	42
Rubber seed (*Hevea brasiliensis*)	1500	79	43
Sago	1200	70†	38
Tapioca	3500	70†	38
Wheat	540	84	40

* Percentage conversion into maltose produced by crystalline sweet potato β-amylase (free from α-amylase and Z-enzyme).

† Completely hydrolysed by a mixture of β-amylase and a β-glucosidase.

[34] Hehre, Carlson, and Hamilton, *J. Biol. Chem.*, 1949, **177**, 289.

[35] Hehre, *ibid.*, p. 267 ; Carlson and Hehre, *ibid.*, p. 281.

[36] Manners, *J.*, 1954, 3527.

[37] Manners and Ryley, *Biochem. J.*, 1955, **59**, 369.

* Apparent percentage conversion into maltose.

† The following analysis is only semi-quantitative, since certain unpurified salivary amylase preparations are contaminated with maltotriase which causes a small increase in Rm, owing to hydrolysis of maltotriose to maltose and glucose.

enzyme action, or to the presence of contaminating branched glucosans. In some cases, the addition of a β-glucosidase, *e.g.*, emulsin or Z-enzyme, to a β-amylolytic digest results in complete saccharification, suggesting that these amyloses contain one or more β-glucosidic linkages which prevent complete β-amylolysis.[38] The nature of the " barriers " to β-amylase in other samples of amylose has not yet been determined.

The main product of β-amylolysis of amylose is maltose (70—100%), whereas amylopectin gives maltose (40—70%) and a β-dextrin of high molecular weight. The determination of the β-amylolysis limit and the nature of the end-products of enzyme action is therefore a convenient method for differentiating between amylose and amylopectin-type polysaccharides even though the chain length of the polysaccharide is unknown. Examples of this type of analysis are given in Table 3.

TABLE 3. *β-Amylolysis limits of* α-1 : 4-*glucosans*

Sample	β-Amylolysis limit	Structural type	Ref.
Clostridium butyricum " amylopectin " (s)	57—71	Branched	44
Neisseria perflava " amylopectin " (cs)	35—40	Branched	35
Corynebacterium diphtheriæ starch (g)			
Fraction A	85—86	Linear	35
Fraction B	64—67	Branched	35
Cryptococcus neoformans (*Torula histolytica*) " amylose "	> 86	Linear	34
Synthetic glucosan (potato amylose and potato Q-enzyme)	58	Branched	45
Synthetic glucosan (potato amylose and bean Q-enzyme)	55	Branched	46
Synthetic glucosan (potato amylose and Q-enzyme from *Polytomella cæca*)	33—34	Branched	47
Polytomella cæca starch			
Fraction V	89	Linear	48
Fraction VI	52	Branched	48
Synthetic " amylose " (glucose 1-phosphate and muscle phosphorylase)	97	Linear	49

(s) Synthesised from sucrose.
(cs) Synthesised by cell-free system from sucrose.
(g) Synthesised from glucose 1-phosphate.

[38] Peat, Pirt, and Whelan, *J.*, 1952, 705, 714; Peat, Thomas, and Whelan, *J.*, 1952, 722.
[39] Potter, Hassid, and Joslyn, *J. Amer. Chem. Soc.*, 1949, **71**, 4075.
[40] Potter, personal communication.
[41] Nussenbaum and Hassid, *J. Biol. Chem.*, 1951, **190**, 673.
[42] Bell and Manners, *J.*, 1952, 3641.
[43] Greenwood and Robertson, *J.*, 1954, 3769.
[44] Hobson and Nasr, *J.*, 1951, 1855.
[45] Barker, Bourne, Peat, and Wilkinson, *J.*, 1950, 3022.
[46] Hobson, Whelan, and Peat, *J.*, 1950, 3566.
[47] Bebbington, Bourne, and Wilkinson, *J.*, 1952, 246.
[48] Bourne, Stacey, and Wilkinson, *J.*, 1950, 2694.
[49] Hassid, Cori, and McCready, *J. Biol. Chem.*, 1943, **148**, 89.

The exterior chain lengths of branched α-1 : 4-glucosans can be calculated from the β-amylolysis limit and chain length, as shown in Table 4. The mean lengths of the interior chains can then be obtained. The lengths of the exterior chains cannot be determined, as yet, by any chemical method.

TABLE 4. *Determination of exterior and interior chain lengths of α-1 : 4-glucosans*

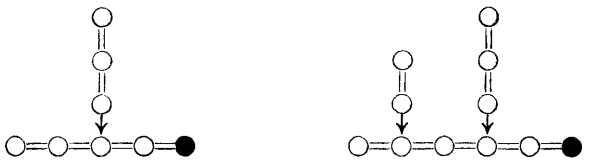
Sample	Chain length	β-L.	E.C.L.	I.C.L.	Ref.
Amylopectins					
Maize	25 (p)	63	18	6	50
Wheat	23 (p)	62	16—17	5—6	50
Easter lily	27 (p)	60	18—19	7—8	50
Tapioca	23 (p)	62	16—17	5—6	50
Sago	22 (p)	62	16	5	50
Potato	27 (p)	59	18—19	7—8	50
Barley	26 (p)	59	18	7	51
Malted barley	17—18 (m)	44	10	6—7	51
Sweet corn 1	12 (p)	47	8	3	52
Sweet corn 2	11 (p)	45	7—8	2—3	52
Waxy maize	22 (m.p.)	53	14	7	53
Waxy sorghum	25 (m.p.)	52	15—16	8—9	53
Glycogens					
Rabbit liver	12—13 (m.p.)	43	8	3—4	42
Rabbit liver	18 (m.p.)	53	12	5	53
Mytilus edulis	16—18 (m.p.)	47	10—11	5—6	42
Helix pomatia	7 (p)	37	5	1	42
Brewer's yeast	13 (p)	44	8	4	54
Baker's yeast	12 (m.p.)	50	8—9	2—3	55
Bacterial and protozoal polysaccharides					
Neisseria perflava	11—12 (m)	57	9	1—2	56
Tetrahymena pyriformis	13 (p)	44	8	4	57
Trichomonas fœtus	15 (p)	60	11—12	2—3	37
Trichomonas gallinæ	9 (p)	51	7	1	37
Bacillus megatherium	11 (m.p.)	46	7—8	2—3	58

β-L. = β-Amylolysis limit.
E.C.L. = Exterior chain length (no. of glucose residues removed by β-amylase + 2·5).
I.C.L. = Interior chain length (chain length − E.C.L. − 1).
p = Periodate oxidation assay. m = Methylation assay.

Use of α-Amylase and R-Enzyme.—By examination of the products produced by the *successive* action of salivary α-amylase and R-enzyme on rabbit-liver glycogen, Whelan and Roberts determined the chain length of

[50] Katz and Potter, personal communication.
[51] Aspinall, Hirst, and MacArthur, unpublished work.
[52] Dvonch and Whistler, *J. Biol. Chem.*, 1951, **181**, 889.
[53] Halsall, Hirst, Hough, and Jones, *J.*, 1949, 3200.
[54] Manners and Maung, *J.*, 1955, 867.
[55] Northcote, *Biochem. J.*, 1953, **53**, 348.
[56] Barker, Bourne, and Stacey, *J.*, 1950, 2884.
[57] Manners and Ryley, *Biochem. J.*, 1952, **52**, 480.
[58] Barry, Gavard, Milhaud, and Aubert, *Ann. Inst. Pasteur*, 1953, **84**, 605.

the polysaccharide, and obtained evidence for a multiply branched structure.[4] α-Amylolysis of the glycogen gave maltose and maltotriose (from the linear portions of the molecule) and a series of branched α-dextrins, which were isolated by charcoal column chromatography. These α-dextrins were then treated with R-enzyme, the 1 : 6-inter-chain linkages being hydrolysed and a series of linear maltosaccharides produced. Since the number of reducing groups liberated by R-enzyme action is dependent on the number of 1 : 6-linkages in the molecule, experimental determination of this number will give the percentage of 1 : 6-linkages and, hence, of non-reducing terminal groups in the molecule. By this method, Whelan and Roberts obtained a chain length of 12·5 for the glycogen ; periodate oxidation gave 13·6. Further examination of the linear maltosaccharides obtained by " debranching " the α-dextrins showed that a small proportion of these were hexa- or hepta-saccharides. The largest maltosaccharide which can arise from a singly branched α-dextrin is maltopentaose ; the



Singly branched α-dextrin. Doubly branched α-dextrin.

FIG. 6

For symbols see Fig. 3. = α-1 : 4-Linkage, resistant to α-amylase.

presence of these higher saccharides suggests that some of the α-dextrins contained two 1 : 6-linkages (see Fig. 6). This finding affords further evidence for multiple branching in glycogen (see also p. 86).

Use of β-Amylase and R-Enzyme.—The successive action of β-amylase and R-enzyme on amylopectin has provided evidence of a multiply branched structure.[33] Methylation studies of amylopectin led Haworth and Hirst [59] to postulate a singly branched " laminated " structure for this polysaccharide, whereas Meyer [60] considered a multiply branched " tree " structure to be the simplest representation of the amylopectin molecule (see Fig. 7). The " laminated " and the " tree " structure differ in the ratio of A : B chains ; in the former, the ratio is $1 : (n - 2)$ where n is the number of chains in the molecule, whereas in the " tree " structure there are approximately equal numbers of A- and B-chains.

β-Amylolysis of an amylopectin, as previously mentioned, is confined to the exterior chains, which are degraded to leave " stubs " composed of two or three glucose residues. Treatment of a β-dextrin with R-enzyme will therefore liberate maltose or maltotriose from the A-chain stubs, whereas B-chains will yield linear saccharides of a much higher molecular weight ; experimentally, the smallest of these has been shown to be maltohexaose. Hence, by determining the amount of maltose and maltotriose

[59] Haworth, Hirst, and Isherwood, *J.*, 1937, 577 ; Haworth, *Nature*, 1947, **160**, 901.
[60] Meyer and Bernfeld, *Helv. Chim. Acta*, 1940, **23**, 875.

produced by the action of R-enzyme on a β-dextrin, it is possible to assess the proportion of A-chains therein, and hence, distinguish between singly branched " laminated " and multiply branched " tree " structures.

Peat and his co-workers[33] treated a β-dextrin of waxy maize starch (of DP *ca.* 3000) with R-enzyme; the observed molar percentage of maltose and maltotriose was 5·3% whereas a singly branched molecule would yield only 0·083%. These authors therefore concluded that " multiple branching is an intrinsic part of the amylopectin molecule ". Hirst and Manners [61] have shown that an amylopectin with a ratio of A : B chains of *ca.* 1 : 4 would yield 5·3% of maltose and maltotriose. Thus, in the waxy maize starch examined by Peat *et al.*, only one unit-chain in every

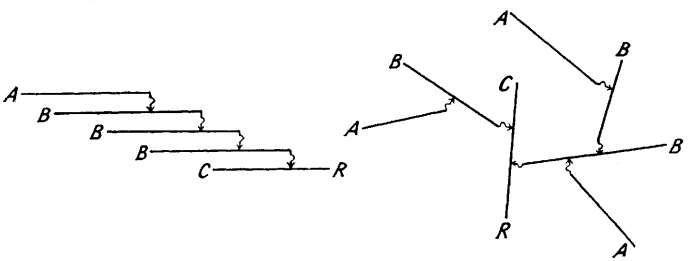


FIG. 7

Singly and multiply branched structures for amylopectin.
For symbols see Fig. 5.

five contained more than one branch point, the molecular structure being therefore intermediate between a " laminated " and " tree " model.

The *combined* action of β-amylase and R-enzyme has been used to determine the unit-chain length of waxy maize starch.[21] A linear chain containing an odd number of glucose residues is degraded by *dilute* solutions of β-amylase into maltose and one molecule of maltotriose, the latter arising from the reducing end of the chain. A polysaccharide sample may be assumed to contain equal numbers of even and odd chains; hence, by treating amylopectin with β-amylase and R-enzyme (to hydrolyse the 1 : 6-linkages which prevent complete β-amylolysis), and determining the amount of maltotriose produced, the number of odd chains in the molecule can be assessed. By this method, waxy maize starch had a chain length of 26; periodate oxidation gave a value of 24—25.

Use of Amylo-1 : 6-Glucosidase and Phosphorylase.—An alternative method of enzymic end-group assay involves the *combined* action of phosphorylase and amylo-1 : 6-glucosidase on amylopectin or glycogen in the presence of inorganic phosphate.[31, 62] Glucose 1-phosphate and glucose are produced; the latter arises from the hydrolysis of 1 : 6-linkages by amylo-1 : 6-glucosidase and, by estimating the molar percentage of glucose the number of 1 : 6-linkages and the chain length can be calculated. This

[61] Hirst and Manners, *Chem. and Ind.*, 1954, 224.
[62] Illingworth, Larner, and Cori, *J. Biol. Chem.*, 1952, **199**, 631.

method has been applied to several samples of amylopectin and glycogen ; the results are in good agreement with those obtained by methylation or potassium periodate oxidation assays.

Stepwise degradation of amylopectin and glycogen by phosphorylase and amylo-1 : 6-glucosidase has provided evidence of multiple branching in these glucosans.[63] If a phosphorylase limit dextrin (LD.1) is treated with amylo-1 : 6-glucosidase and the latter then inactivated, the dextrin will be susceptible to further attack by phosphorylase ; this phosphorolysis,

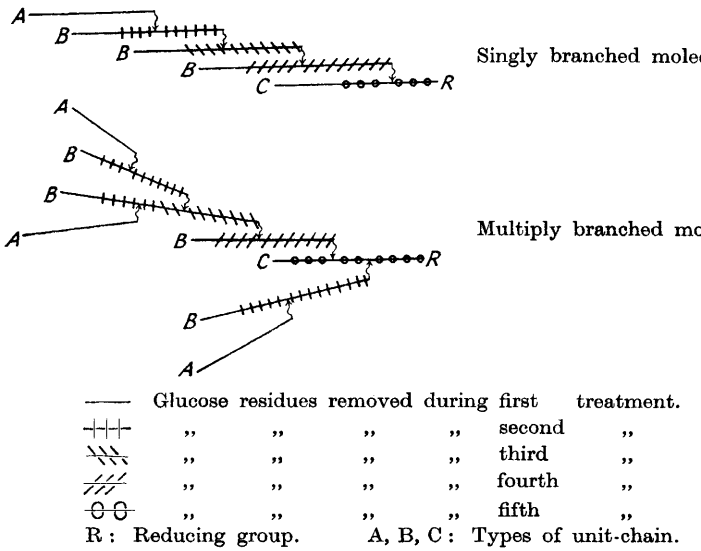


FIG. 8

The stepwise degradation of a singly and a multiply branched molecule by muscle phosphorylase and amylo-1 : 6-glucosidase.

however, is incomplete. A second dextrin (LD.2) can be isolated, and on treatment with amylo-1 : 6-glucosidase, it again becomes susceptible to phosphorylase action. Each successive action of phosphorylase and amylo-1 : 6-glucosidase thus causes the removal of the A-chains from the phosphorylase limit dextrin. Furthermore, about 50% of the branch points are removed during the first combined treatment, but only about 20% during the second. Since a singly branched molecule would lose only one of the branch points at each treatment, the above experiments provide evidence for multiply branched structures (see Fig. 8).

Synergic Reactions.—In 1952 Schwimmer and Garibaldi [22] reported that, although glucose was not a major product of the action of malt α-amylase on starch, the addition of *Bacillus macerans* amylase to an enzymic digest of starch and malt α-amylase increased the rate and extent of hydrolysis and resulted in the formation of considerable amounts of glucose. In the

[63] Larner, Illingworth, Cori, and Cori, *J. Biol. Chem.*, 1952, **199**, 641.

following year, Whelan [21] observed that when glycogen or amylopectin is degraded by a mixture of R-enzyme and α-amylase, the end-products of this action included glucose. These two enzymes acting together also degraded panose (4-α-*iso*maltosyl-D-glucose) to give a mixture of glucose and maltose; neither enzyme acting separately liberated glucose from amylopectin or glycogen, nor attacked panose. It appears, therefore, that certain polysaccharase-substrate "complexes" can be modified by the presence of a second enzyme so that an abnormal reaction occurs.

Enzymic Degradation of Dextrans

Dextrans are considered to be α-1 : 6-glucosans of high molecular weight, usually branched, which are produced extracellularly by certain bacteria (*e.g.*, *Leuconostoc mesenteroides*) when grown in the presence of sucrose.[64] The existence of enzymes capable of hydrolysing these polysaccharides was unknown until 1948, when it was reported that various moulds showed dextranase activity when grown in a medium containing dextran.[65] Similar results were observed with the bacterium *Cellvibrio fulva*.[66] More recently, cell-free extracts of an anaerobic bacterium from the human intestine have been shown to contain dextranase.[67] In an intensive search for dextranases, approximately 200 strains of yeasts, moulds, and bacteria were examined by Tsuchiya and his collaborators; [68] they found that these organisms normally do not produce appreciable amounts of dextranase. However, certain moulds (*e.g.*, *Aspergillus niger* and some strains of *Penicillium*), when grown in submerged culture in the presence of dextran, produce dextranases extracellularly. Dextranase production is therefore an adaptive process.

The first detailed study of dextranase action was made by Jeanes and co-workers [9] who degraded a dextran (synthesised by *L. mesenteroides*) with culture filtrates of two strains of the mould *Penicillium funiculosum*. 95% of the linkages in the dextran were of the α-1 : 6-type. One dextranase gave *iso*maltose (45%), *iso*maltotriose (22%), and higher saccharides, whilst the second dextranase yielded glucose (10%), *iso*maltose (53%), *iso*maltotriose (7%), and a series of higher saccharides; separation of these sugars was effected by charcoal column chromatography.

Dextrans from various strains of *L. mesenteroides*, which contain different proportions of α-1 : 6-glucosidic linkages, are hydrolysed at different rates by a particular dextranase.[68] Since adaptively formed dextranases are free from other polysaccharases,[66] the presence in the dextran of linkages other than α-1 : 6 will interfere with hydrolysis; the relative rates of hydrolysis of different dextrans by a dextranase may therefore be used qualitatively to detect structural differences in these polysaccharides.

[64] For reviews of dextran chemistry see Stacey and Ricketts, *Fortschr. Chem. Org. Naturstoffe*, 1951, **8**, 28; Evans and Hibbert, *Adv. Carbohydrate Chem.*, 1946, **2**, 209.

[65] Nordström and Hultin, *Svensk Kem. Tidskr.*, 1948, **60**, 283; Hultin and Nordström, *Acta Chem. Scand.*, 1949, **3**, 1405.

[66] Ingelman, *ibid.*, 1948, **2**, 803.

[67] Hehre and Sery, *J. Bact.*, 1952, **63**, 424.

[68] Tsuchiya, Jeanes, Bricker, and Wilham, *ibid.*, 1952, **64**, 513.

Furthermore, quantitative studies similar to those of Jeanes and co-workers should enable the inter-chain linkages to be identified. Recent chemical studies [69] have indicated that certain dextrans contain 1 : 3-linkages, in addition to, or in place of, the 1 : 4-linkages previously reported ; accordingly, further work in this field is necessary.

From a study of the change in viscosity of dextran during enzymic degradation, Hultin and Nordström,[65] and Ingelman,[66] have concluded that dextranase catalyses a random rather than a stepwise hydrolysis of the polysaccharide. The isolation of glucose, *iso*maltose, and *iso*maltotriose from a dextran–dextranase digest [9] confirms this view. Accordingly, dextranases may have considerable importance as a means of partially degrading dextrans to the molecular-weight range required for use as a blood-plasma substitute.[66] It may also be possible to use certain dextranases to hydrolyse the α-1 : 6-linkages in amylopectin or glycogen, since only a few such " debranching " enzymes are known.

Enzymic Degradation of β-Glucosans

Cellulose.—Cellulose, the main constituent of plant cell walls, is composed essentially of linear chains, each consisting of several thousand β-1 : 4-linked D-glucopyranose residues.[70] In view of the great industrial importance of this natural polymer, studies of the hydrolytic enzymes which degrade cellulose will have considerable industrial significance in addition to physiological interest. Cellulases are widely but erratically distributed in Nature ; they are present in certain higher plants (*e.g.*, barley) and in invertebrate digestive secretions, and are produced extracellularly by many bacteria and fungi ; they are absent from mammalian digestive secretions, although herbivores maintain populations of symbiotic micro-organisms in their intestinal tracts, which produce cellulases.[8, 71]

In general, cellulases have not been extensively purified, and our knowledge of the mechanism of enzyme action is accordingly limited. Many cellulase preparations show cellobiase activity ; separation of these enzymes has been achieved by Grassmann and his collaborators [72] who removed cellobiase from *Aspergillus oryzæ* cellulase by selective adsorption of the former on aluminium hydroxide, and by Whistler and Smart [73] who used columns of powdered cellulose to adsorb cellobiase from an *Aspergillus niger* cellulase preparation. An important advance in this field has been made by Whitaker who has purified the cellulase from the mould *Myrothecium verrucaria* ; [74] the purified enzyme was homogeneous in the ultra-centrifuge and on electrophoresis, and has a molecular weight of 63,000.[75]

[69] Barker, Bourne, Bruce, Neely, and Stacey, *J.*, 1954, 2395 ; Sloan, Alexander, Lohmar, Wolff, and Rist, *J. Amer. Chem. Soc.*, 1954, **76**, 4429.

[70] See Aspinall, *Biochem. Soc. Symp.*, 1953, **11**, 42.

[71] Pigman in " The Enzymes ", by Sumner and Myrbäck, Academic Press, New York, 1951, Vol. I, Part 2, p. 728.

[72] Grassmann, Zechmeister, Tóth, and Stadler, *Annalen*, 1933, **503**, 167.

[73] Whistler and Smart, *J. Amer. Chem. Soc.*, 1953, **75**, 1916.

[74] Whitaker, *Arch. Biochem. Biophys.*, 1953, **43**, 253.

[75] Whitaker, Colvin, and Cook, *ibid.*, 1954, **49**, 257.

Since the natural substrate for cellulases is insoluble in water, enzyme action cannot be determined viscometrically; in view of the simplicity and convenience of such methods, soluble derivatives of cellulose (*e.g.*, carboxymethylcellulose) have been used as substrates in many cases. The latter are hydrolysed *ca.* 10—30 times more rapidly than cellulose, under identical conditions, as shown by measurements of reducing power, the actual ratio depending on the enzyme source.[8] This variation in the relative rates of hydrolysis of cellulose and its soluble derivatives has led to the postulation of a multienzyme mechanism for cellulase action. According to Reese and his co-workers [76] cellulase preparations contain an enzyme (C_1) which alters the physical state of cellulose fibres and liberates the polymeric chains, whilst a second enzyme (Cx) catalyses the hydrolysis of these chains to reducing sugars. The enzyme Cx, acting alone, is supposed to be able to degrade carboxymethylcellulose but not native cellulose. The ratio of the rates of hydrolysis of these substrates would therefore depend on the relative concentrations of C_1 and Cx in a particular cellulase preparation. Quantitative data to test the validity of this hypothesis have, so far, been obtained only with the cellulase from *Myrothecium verrucaria*.[74] This cellulase showed the same activity towards five substrates ranging in degree of polymerisation (and hence solubility) from cellulose to cellobiose, throughout purification from the crude culture filtrate of this mould, to the final purified state.

A recent observation by Whitaker [77] on the effect of proteins on *M. verrucaria* cellulase is of considerable interest. He showed that cellulase action on insoluble substrates is stimulated by the addition of small amounts of certain proteins (*e.g.*, bovine plasma albumin and β-lactoglobulin) which are adsorbed on the cellulose. No such effect was obtained with a soluble substrate. The mechanism of this "activation" is, as yet, unknown, but the apparent differences in activity of various "cellulases" towards soluble and insoluble cellulose may well have been due to the presence of protein impurities in the various enzyme preparations, rather than to the presence of different proportions of two distinct cellulolytic enzymes.

The nature of the end-products of cellulase action has been investigated by several workers. Whistler and Smart [73] have isolated glucose and cellobiose from the hydrolysate of cellulose by *A. niger* cellulase. Sugars other than these were absent, and cellobiose was not formed on prolonged incubation of glucose with the enzyme; hence the cellobiose was preformed in the substrate. Whether glucose is a primary product of *A. niger* cellulase action or arises solely from cellobiose (the cellulase contained traces of cellobiase) is not yet known. The products of the action of *Clostridium thermocellulaseum* cellulase are glucose, cellobiose, and probably cellotriose; [78] in this case, the glucose is a primary product of cellulase action. Whitaker [74] has shown that *M. verrucaria* cellulase liberates both glucose

[76] Reese, Siu, and Levinson, *J. Bact.*, 1950, **59**, 485; Reese and Levinson, *Physiologia Plantarum*, 1952, **5**, 345. [77] Whitaker, *Science*, 1952, **116**, 90.

[78] Enebo, "Studies in Cellulose Decomposition by an Anaerobic Thermophilic Bacterium and Two Associated Non-Cellulolytic Species", Stockholm, 1954.

and cellobiose—in roughly equimolar proportions—from both a soluble and an insoluble cellulose. This cellulase, and that from *Cl. thermocellulaseum*, thus catalyse random hydrolytic cleavage of the β-1 : 4-linkages in cellulose. In contrast, cellobiose is the sole product from the degradation of " hydrocellulose " by a purified cellulase from the fungus *Irpex lacteus* and enzyme action is believed to involve the release of cellobiose molecules from the ends of long cellulose chains.[79] It is evident that the term " cellulase " comprises a group of closely related enzymes, and that differences in action pattern exist between cellulases from different organisms. The recent demonstration [80] that cell-free filtrates of certain cellulolytic moulds contain several chromatographically distinct cellulases is particularly significant.

Cellulase action *in vitro* is usually incomplete. Apart from the heterogeneous nature of the action, the physical state of the substrate is of prime importance. The surface potential of cellulose, upon which depends the rate of formation of the enzyme–substrate complex, is an important factor—as shown by the stimulating effect on cellulase action, at certain pH's, of basic dyes (but not acidic dyes) which are adsorbed on the substrate.[81] Furthermore, the degree of crystallinity of the cellulose is important, since the available evidence indicates that cellulase activity is limited to the amorphous regions where the cellulose chains in the fibre are more readily accessible.[82]

Cellulases are a group of enzymes of considerable importance. Attempts have been made to use cellulose breakdown and fermentation as an industrial method for the production of alcohols and acids, and as a means of saccharifying plant fibres.[78] In the laboratory, cellulases provide a method for investigating the structural relations between cellulose, the hemicelluloses, and lignin in woody plant tissues.

Lichenin, Laminarin, and Glucan.—Extracts of some higher plants (*e.g.*, barley [42, 83]), seaweeds,[10] and snail secretions [84] contain enzyme systems which hydrolyse the β-glucosans lichenin, laminarin, and glucan (yeast " cellulose "). Early investigations in this field were hampered by lack of structural knowledge of these substrates ; recent chemical studies have now shown lichenin to be a linear β-glucosan containing both 1 : 3- and 1 : 4-linkages,[85] laminarin to be *essentially* a linear β-1 : 3-glucosan,[86] and glucan to be a branched β-1 : 3-glucosan containing *ca.* 11% of β-1 : 2-interchain linkages.[87]

[79] Nishizawa and Kobayashi, *Symp. Enzyme Chem.* (*Japan*), 1953, **8**, 123 ; *Chem. Abs.*, 1953, **47**, 12438.

[80] Jermyn, *Austral. J. Sci. Research*, 1952, *B*, **5**, 433 ; Reese and Gilligan, *Arch. Biochem. Biophys.*, 1953, **45**, 74.

[81] Basu and Whitaker, *ibid.*, 1953, **42**, 12.

[82] Cf. Nickerson, *Adv. Carbohydrate Chem.*, 1950, **5**, 103.

[83] Dillon and O'Colla, *Chem. and Ind.*, 1951, 111.

[84] Pigman, ref. 71, p. 725—744.

[85] Meyer and Gürtler, *Helv. Chim. Acta*, 1947, **30**, 751 ; Boissonnas, *ibid.*, p. 1703 ; N. B. Chanda, Ph.D. Thesis, Edinburgh, 1952.

[86] Connell, Hirst, and Percival, *J.*, 1950, 3494 ; Percival and Ross, *J.*, 1951, 720,

[87] Bell and Northcote, *J.*, 1950, 1944.

The above-mentioned extracts are known to contain several enzymes (*e.g.*, cellulase and β-glucosidase) in addition to the enzyme(s) responsible for the β-1 : 3-glucosanase activity.

Lichenase and cellulase are distinct enzymes, as shown by their different pH optima (5·9 and 4·7 respectively for fungal enzymes) and by the separation of the two activities by fractional precipitation with ether and ethanol.[88] The end-product of snail lichenase action (of unestablished homogeneity) is glucose;[89] similar information with purified lichenases from other sources is not yet available.

The enzymic hydrolysis of laminarin has been studied by several workers. From a hydrolysate with snail juice, Barry[90] isolated laminaribiose (3-β-D-glucopyranosyl-D-glucose)—further evidence for the presence of β-1 : 3-glucosidic linkages in laminarin. Plant enzymes hydrolyse laminarin initially to a mixture of glucose, laminaribiose, and higher oligosaccharides; the final end-product is glucose.[83] In marine algæ, at least two enzymes are involved in this action; one enzyme hydrolyses in random manner non-terminal β-1 : 3-linkages, yielding a series of oligosaccharides, whilst a second enzyme hydrolyses terminal β-linkages, thereby producing glucose.[10]

Yeast glucan is hydrolysed by almond emulsin and seaweed extracts to give glucose,[10] and by cereal extracts to give a mixture of glucose and laminaribiose, thus confirming the configuration of the glucosidic linkages.[83]

Until complete separation of these β-glucosanases has been achieved, little progress can be made in enzymic investigations of the fine structure of lichenin and other β-glucosans, since their action patterns are, as yet, unknown. In particular a specific β-1 : 3-glucosanase would be of value in detecting the presence of linkages other than β-1 : 3 in laminarin, in view of the reported presence of gentiobiose and 1-β-glucosylmannitol in partial acid hydrolysates of this polysaccharide.[91]

Enzymic Degradation of Hemicelluloses

Mannans.—Present knowledge of the enzymic degradation of mannans is limited and, although mannanase (mannase) activity has been reported in a number of protein preparations, these enzymes have not been purified or characterised.

Mannanases appear to be erratically distributed in Nature; they occur in malt,[92] in the culture filtrate of an agar-agar splitting bacterium,[93] in the mould *Neurospora sitophila*,[94] and in the marine alga *Cladophora rupestris*.[10] The mould *Chætomium globosum* can synthesise mannanase adaptively.[95]

[88] Freudenberg and Ploetz, *Z. physiol. Chem.*, 1939, **259**, 19.

[89] Karrer and Staub, *Helv. Chim. Acta*, 1924, **7**, 518.

[90] Barry, *Sci. Proc. R. Dublin Soc.*, 1941, **22**, 423; laminaribiose has been synthesised by Bächli and Percival, *J.*, 1952, 1243.

[91] Peat, Whelan, and Lawley, *Biochem. J.*, 1953, **54**, xxxiii; *Chem. and Ind.*, 1955, 35.

[92] Klages and Maurenbrecher, *Annalen*, 1938, **535**, 175.

[93] Ishimatsu and Kibesaki, *Symp. Enzyme Chem. (Japan)*, 1950, **4**, 75; *Chem. Abs.*, 1952, **46**, 2124.

[94] Takai, *Jap. J. Nutrition*, 1950, **8**, 131; *Chem. Abs.*, 1951, **45**, 8058.

[95] Sørensen, *Physiologia Plantarum*, 1952, **5**, 183; *Nature*, 1953, **172**, 305.

The algal mannanase appears to catalyse random hydrolytic cleavage of ivory-nut mannan A (essentially a β-1 : 4-mannosan [96]) since the end-products include mannose, mannobiose, and other saccharides. It has no action on the branched yeast mannan. Ivory-nut mannan is also hydrolysed by a malt extract to give a mixture of mannose and a disaccharide (presumably mannobiose); [92] this extract contains two enzymes—a true mannanase and a mannobiase, since mannose is the sole end-product from the digestion of salep mannan by a normal extract, whereas an aged preparation yields only the disaccharide.[97] Salep mannan is known to be a 1 : 4-mannosan,[98] and the above findings suggest that the mannosidic linkages have a β-configuration.

Galactomannans.—Studies on the enzymic degradation of galactomannans have so far been limited to gum gatto (carubin [99]) and guaran; [100] in both cases, information on the structure of the polysaccharide has been obtained.

Gum gatto, the galactomannan from the carob bean *Ceratonia siliqua*, contains D-mannose residues (*ca.* 84%) together with D-galactose (*ca.* 16%).[101] Methylation studies indicate that it comprises a main chain of 1 : 4-linked mannose units and that one-fifth of these have a single galactose residue attached at $C_{(6)}$. It has been degraded [99] by a commercial polysaccharase preparation "Helisol" which contains an alkali-labile mannanase and an alkali-stable galactosidase. At alkaline pH's, galactose is liberated, but the viscosity of gum gatto is only slightly reduced, whereas at more acid pH's the viscosity quickly decreases, with the liberation of a mixture of reducing substances; these include galactomannose saccharides, mannose-containing oligosaccharides, and free galactose. These findings are in accord with the structure suggested for the gum. Since certain mannanase preparations attack both ivory-nut mannan and gum gatto, it is probable that the mannose residues in the latter also have a β-configuration.

Guaran, a reserve polysaccharide found in the endosperm of guar seeds, on acid hydrolysis gives D-mannose (64%) and D-galactose (36%)[102]. Chemical studies have shown that guaran consists essentially of a chain of 1 : 4-linked D-mannose residues; to alternate residues are attached, at $C_{(6)}$, single D-galactose residues.[102] The characterisation of the oligosaccharides produced during the enzymic hydrolysis of guaran has enabled the identity of the constituent glycosidic linkages to be confirmed.

An enzyme preparation from germinated guar seeds brought about 65% hydrolysis of the guaran. The hydrolysate was fractionated by a charcoal-chromatographic procedure and found to contain the following sugars:

[96] Aspinall, Hirst, Percival, and Williamson, *J.*, 1953, 3184.

[97] Pringsheim and Genin, *Z. physiol. Chem.*, 1924, **140**, 299.

[98] Klages and Niemann, *Annalen*, 1936, **523**, 224.

[99] Deuel, Leuenberger, and Huber, *Helv. Chim. Acta*, 1950, **33**, 942.

[100] Whistler and Stein, *J. Amer. Chem. Soc.*, 1951, **73**, 4187; Whistler and Smith, *ibid.*, 1952, **74**, 3795.

[101] Hirst and Jones, *J.*, 1948, 1278.

[102] Heyne and Whistler, *J. Amer. Chem. Soc.*, 1948, **70**, 2249; Ahmed and Whistler, *ibid.*, 1950, **72**, 2524; Palmer and Ballantyne, *ibid.*, p. 736.

monosaccharides (65% including most of the galactose originally present), mannobiose (7%), a galactomannose (0·5%), and a mannotriose (7·5%).[100] No higher saccharides were produced by incubation of the enzyme with mannose. Methylation experiments showed that the mannosidic linkages in mannobiose and mannotriose were of the β-1 : 4-type. Guaran must therefore contain a main chain of β-1 : 4-linked D-mannose units. The galactomannose was shown to be 6-α-D-galactopyranosyl-β-D-mannopyranose —further confirmation for the presence of galactose residues attached to $C_{(6)}$ of mannose residues in the main chain of guaran.

Xylans.—Little information is available at present on the enzymes catalysing the hydrolytic degradation of xylans. Xylanase activity has been reported in extracts of germinated barley,[84] in snail secretions,[84] in moulds (*e.g.*, *Aspergillus niger* and *A. oryzæ*) and in extracts of some seaweeds.[10]

Sørensen [95] has shown that the mould *Chætomium globosum* can produce a xylanase when grown in a medium containing xylan (*i.e.*, it is an "adaptive" enzyme). This "xylanase" has optimal activity at pH 6·5 (cf. pH 4·6 for barley xylanase [103]), and appears to contain two components—an enzyme capable of hydrolysing xylan in random fashion, and a less stable enzyme which hydrolyses xylobiose. The combined action of these enzymes produces xylose and a series of oligosaccharides, whilst the former liberates only oligosaccharides from xylan. A related enzyme system occurs in certain seaweeds ; [10] esparto xylan (a β-1 : 4-xylosan [104]) is degraded by the algal xylanases to give a mixture of xylose, xylobiose, and xylotriose ; the same enzyme preparation will also slowly hydrolyse xylobiose and xylotriose.

Enzymic studies have yielded information on the structure of hemicellulose A of oak wood,[105] and a wheat-straw xylan.[95] Hemicellulose A of oak sapwood, on treatment with Taka-diastase (a commercial preparation containing several polysaccharases), yielded glucose and a "xylan" which was identical with that found in the heartwood.[105] Prolonged incubation of this "xylan" with Taka-diastase gave xylose (3 parts) and a water-soluble polysaccharide (2 parts) ; on acid hydrolysis, the latter yielded xylose and a monomethylhexuronic acid, and analysis indicated these sugars to be present in the molar ratio of 6 : 1. An enzyme-resistant "core" has also been prepared from wheat-straw xylan.[95] Incubation of this xylan with a mould xylanase (from *Chætomium globosum*) gave xylose, a small quantity of arabinose, and a water-soluble enzyme-resistant polysaccharide which was composed of xylose, arabinose, glucose, and uronic acids. Methylation studies of such enzyme-resistant "cores" would be of great interest, provided the homogeneity of the xylan is established.

To summarise, the widespread use of hemicellulases as "analytical tools" must await further separation and purification of the individual enzymes and the determination of their action patterns. An interesting

[103] Voss and Butter, *Annalen*, 1938, **534**, 161, 185.
[104] Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.
[105] O'Dwyer, *Biochem. J.*, 1939, **33**, 713.

example of hemicellulase action has been reported by Bishop and Whitaker [105a] who have obtained a series of arabinose–xylose oligosaccharides by hydrolysing wheat-straw xylan with an enzyme preparation from *Myrothecium verrucaria*. This observation is further evidence for the existence of arabinose as a minor constituent of wheat-straw xylan.

Enzymic Degradation of Fructosans

Although several investigations of the enzymic degradation of inulin (essentially a β-1 : 2-fructosan [1]) have been reported, little attention has been paid to the enzymic breakdown of other fructosans.

An early investigation by Pringsheim and Ohlmeyer [106] showed that extracts of the mould *A. niger* had inulinase (inulase) activity; complete degradation of the inulin occurred, with the liberation of fructose (over 90%) and glucose (1·5%). These extracts also hydrolysed sucrose. For some time it appeared that inulinase and invertase were identical enzymes, but recent findings are in conflict with this hypothesis. Hudson and his collaborators [107] studied the action of purified yeast "invertase" on sucrose and inulin; the optimum pH's were found to be 5·2 and 3·6 respectively, suggesting the presence of two distinct enzymes. Furthermore, a comparative study of the inulinase activity of several "invertase" preparations revealed large differences in the ratios of the two activities. More recently Legrand and Lewis [108] have obtained a yeast inulinase preparation devoid of invertase activity and a yeast invertase which had no action on asphodeloside (an inulin-type fructosan), whilst Edelman and Bacon [109] have shown that extracts of Jerusalem artichokes, which have relatively little invertase activity, rapidly hydrolyse inulin.

Inulinases have been detected in a variety of sources including yeast,[110] moulds (*e.g.*, *Penicillium notatum* [111]), and those plants which store inulin as a carbohydrate reserve.[112]

A study of inulinase action has provided evidence of a glucofructosan structure for inulin.[7] Inulinase from the mould *Sterigmatocystis nigra* hydrolysed inulin to give, at intermediate stages of hydrolysis, a series of glucofructosyl oligosaccharides (D, C, B) in accordance with the scheme:

$$D + H_2O \longrightarrow C + \text{fructose} \qquad C + H_2O \longrightarrow B + \text{fructose}$$
$$B + H_2O \longrightarrow \text{Sucrose} + \text{fructose} \qquad \text{Sucrose} + H_2O \longrightarrow \text{glucose} + \text{fructose}$$

Enzyme action therefore involves a stepwise hydrolysis of β-1 : 2-fructofuranosidic linkages, beginning from the non-reducing terminal fructose group, and this experiment provides evidence that inulin is a linear molecule

[105a] Bishop and Whitaker, *Chem. and Ind.*, 1955, 119.
[106] Pringsheim and Ohlmeyer, *Ber.*, 1932, **65**, 1242; 1933, **66**, 1292.
[107] Adams, Richtmyer, and Hudson, *J. Amer. Chem. Soc.*, 1943, **65**, 1369.
[108] Legrand and Lewis, *Compt. rend.*, 1951, **232**, 1439.
[109] Edelman and Bacon, *Biochem. J.*, 1951, **49**, 446.
[110] Hongô, *J. Agric. Chem. Soc. Japan*, 1942, **18**, 981; *Chem. Abs.*, 1951, **45**, 4753.
[111] Mori, *J. Japan Soc. Food Nutrition*, 1951, **3**, 209; *Chem. Abs.*, 1952, **46**, 7139.
[112] Shibuya and Tsukamoto, *J. Japan Biochem. Soc.*, 1950, **22**, 189; *Chem. Abs.*, 1951, **45**, 9090.

composed of β-1 : 2-fructofuranose residues, at the reducing end of which is attached a single α-glucosyl residue.

Holden and Tracey [113] have reported that the digestive juices of the snails *Helix pomatia* and *H. aspersa* hydrolyse inulin, grass and bacterial levans (which contain 2 : 6-fructofuranosidic linkages [1]), and irisin; no purification or separation of these activities was recorded. The hydrolysis of grass levan and irisin by extracts of Jerusalem artichoke is incomplete, only *ca.* 20% of the fructoside linkages being broken.[109] Since irisin contains both 1 : 2- and 2 : 6-fructofuranosidic linkages,[1] further enzymic studies should enable the branched nature of this fructosan to be characterised.

The existence of an enzyme catalysing the hydrolysis of bacterial levan has been reported recently.[114] The enzyme (levanpolyase) is produced by levan-forming bacteria, *e.g.*, *Azotobacter chroöcoccum*, when grown on levan; enzyme action is incomplete, the main product being fructosaccharides of mean DP 11. If the same bacteria are grown on sucrose, the culture fluids contain levanpolyase and a second enzyme "levanoligase" which hydrolyses the fructosaccharides to fructose. Levanoligase, but not levanpolyase, also hydrolyses sucrose. Since the enzymic hydrolysis of 2 : 6-fructofuranosidic linkages is uncommon, details of the action pattern of these adaptively produced bacterial enzymes will be awaited with interest.

Enzymic Degradation of Pectic Substances

The pectic substances comprise a group of closely related plant polysaccharides which are polymers of galacturonic acid; the carboxyl groups of the acid residues may be partly neutralised by bases, or may be partly esterified by methyl groups.[115] The most important pectic substance is pectin, a water-soluble polygalacturonide which usually contains about 12% of ester methoxyl and forms gels with sugar and acids under suitable conditions. Pectin occurs in many fruits and vegetables, and is prepared on an industrial scale from citrus fruits. Pectic acid is produced by the de-esterification of pectin with acid or alkali, or with the enzyme pectinesterase (PE; also known as pectase). Chemical studies have shown that the galacturonide linkages in pectic acid are of the α-1 : 4-type.

Pectinesterase occurs in higher plants (*e.g.*, tomato, citrus fruits) and in certain moulds.[116, 117] In the latter sources it is usually associated with polygalacturonase (PG; also known as pectinase), an enzyme catalysing the hydrolysis of the glycosidic linkages in pectic acid but having no action on glycosidic or methyl ester linkages in pectin. Pectinesterase activity may be followed either by determination of the liberated methanol, or by continuous titration of the liberated carboxyl groups with dilute alkali.

[113] Holden and Tracey, *Biochem. J.*, 1950, **47**, 407.

[114] Hestrin and Goldblum, *Nature*, 1953, **172**, 1046.

[115] For reviews see Hirst and Jones, *Adv. Carbohydrate Chem.*, 1946, **2**, 235; Kertesz, "The Pectic Substances", Interscience Publ., New York, 1951; McCready and Owens, *Econ. Bot.*, 1954, **8**, 29.

[116] Kertesz and McColloch, *Adv. Carbohydrate Chem.*, 1949, **5**, 79.

[117] Kertesz, ref. 71, p. 745.

The pure enzyme does not depolymerise pectin and has no action on methyl galacturonate or methyl galacturonide methyl ester. Enzymic demethylation of pectin is rapid, and appears to take place by a stepwise rather than a random action, in contrast to acid or alkali demethylation. Differences have been reported in the physical and chemical properties of pectinesterases from different sources; thus mould pectinesterase has an optimum pH range of 4·5—5·0 and is very thermolabile, whilst tomato pectinesterase is most active at pH 7—8 and is more resistant to inactivation by heat.[118]

Several enzymes are now known which can hydrolyse the α-1 : 4-galacturonide linkages in pectic substances. Recent work has established the existence of two main groups of such enzymes: (*a*) polygalacturonases (PG), which degrade pectic acid to the disaccharide level; and (*b*) pectin depolymerases (PD),* which degrade pectin and pectic acid to polyuronides of low molecular weight. These polyuronides are however susceptible to attack by polygalacturonase. In addition, certain polygalacturonase preparations may contain a galacturonidase capable of hydrolysing di- and tri-galacturonic acid to galacturonic acid.[119]

Polygalacturonase activity has been detected in several moulds, in certain bacteria, in barley malt, and in snail digestive juices.[116, 117] In the mould *Aspergillus fœtidus*, polygalacturonase is associated with a pectin depolymerase.[119] Fungal polygalacturonase has been purified by several workers, and has been widely studied. It is a highly specific enzyme; it does not attack pectin or the Pneumococcus Type I polysaccharide (which contains 60% of α-1 : 4-D-galacturonic acid residues).[120] It catalyses random hydrolysis of pectic acid, the viscosity of the substrate being halved at only 2% hydrolysis, and at intermediate stages of hydrolysis, tri-, tetra-, and penta-galacturonic acids are produced.[121]. The composition of the end-products of polygalacturonase action appears to depend on the particular enzyme preparation used; di- and tri-galacturonic acid are the main products when an *Aspergillus fœtidus* preparation is used,[119] whereas other purified fungal polygalacturonases yield galacturonic acid as the sole product.[11] The rates and extents of hydrolysis of three pectic acids ranging in molecular weight from 1900 to 35,000 have been found to be identical.[11] A limited number of yeasts (*e.g.*, *Saccharomyces fragilis*) produce an exocellular polygalacturonase which resembles the fungal enzyme in that the extent of hydrolysis of partially demethylated pectins is inversely proportional to the methoxyl content of the pectin; it differs, however, since the hydrolysis of pectic acid is incomplete, the end-products being mono- and di-galacturonic acids.[122] Physicochemical studies of purified yeast

[118] McColloch and Kertesz, *Arch. Biochem.*, 1947, **13**, 217; Calesnick, Hills, and Willaman, *ibid.*, 1950, **29**, 432.

[119] Ayres, Dingle, Phipps, Reid, and Solomons, *Nature*, 1952, **170**, 834.

[120] Lineweaver, Jang, and Jansen, *Arch. Biochem.*, 1949, **20**, 137.

[121] Altermatt and Deuel, *Helv. Chim. Acta*, 1952, **35**, 1423; Rahman and Joslyn, *Food Research*, 1953, **18**, 308.

[122] Luh and Phaff, *Arch. Biochem. Biophys.*, 1954, **48**, 23; Demain and Phaff, *J. Biol. Chem.*, 1954, **210**, 381.

* This abbreviation is preferable to DP as suggested by Kertesz (ref. 117).

polygalacturonase support the hypothesis that the activity is due to a single enzyme.[123]

Certain fungal polygalacturonase preparations appear to be heterogeneous. Schubert [124] observed that culture extracts of *Aspergillus niger* contained four different polygalacturonase enzymes, which were differentiated by selective inactivation or selective adsorption. Full details of the specificity of these four enzymes were not recorded. Polygalacturonase preparations from *Aspergillus fœtidus* contain two enzymes; one of these appears to be a type of pectin depolymerase since it degrades pectin or pectic acid to polyuronides of low molecular weight, whilst the second enzyme shows true polygalacturonase activity in that it degrades pectic acid (and the polyuronides of low molecular weight) to a mixture of di- and trigalacturonic acid.[119, 125]

The existence of pectin-depolymerases has only recently been verified, although their presence in pectolytic preparations has been suspected for some time. Thus, pectolytic preparations from tomatoes contain an enzyme which differs from fungal polygalacturonase in that it is relatively insensitive to heat, has an optimum pH of 4·5 (that of fungal polygalacturonase is 3·5), and causes only partial breakdown of pectic acid, the end-products being polyuronides of DP > 5.[116, 126] The mould *Neurospora crassa* produces an extracellular enzyme which partly degrades pectin and pectic acid; [127] the end-product of this action, at *ca.* 30% hydrolysis, is a polygalacturonide of molecular weight 4000. A similar depolymerising enzyme has been obtained from a commercial enzyme preparation and named polymethylgalacturonase (PMG).[128] Purified polymethylgalacturonase, which has negligible pectinesterase activity, catalyses a 26% hydrolysis of pectin and pectic acid. Enzyme action was random, since at 0·5% hydrolysis the viscosity of the substrates was halved. The end-product of this action had $[\alpha]_D + 221°$ (those of pectin and galacturonic acid are $+ 235°$ and $+ 56°$ respectively), and was immobile on a paper chromatogram. Cell-free extracts of *Bacterium aroideæ* also contain a depolymerising enzyme which rapidly reduces the viscosity of pectic acid or pectin (OMe 9%) but with little increase in reducing power.[129] Pectin depolymerases thus differ from polygalacturonases in (*a*) ability to degrade pectin without previous de-esterification, (*b*) inability to effect complete hydrolysis, (*c*) chemical and physical properties, *e.g.*, optimum pH.

It is now apparent that the field of pectolytic enzymes is as complex as that of amylolytic enzymes, and future studies must be directed towards the preparation of homogeneous enzymes which are essential for detailed investigations of enzyme action.

[123] Demain and Phaff, *Nature*, 1954, **174**, 515.
[124] Schubert, *Nature*, 1952, **169**, 931.
[125] Dingle, Reid, and Solomons, *J. Sci. Food Agric.*, 1953, **4**, 149.
[126] McColloch and Kertesz, *Arch. Biochem.*, 1948, **17**, 197.
[127] Roboz, Barratt, and Tatum, *J. Biol. Chem.*, 1952, **195**, 459.
[128] Seegmiller and Jansen, *ibid.*, p. 327.
[129] Wood, *Nature*, 1951, **167**, 771.

Summary and Conclusions

In this Review, present knowledge of the enzymes catalysing the hydrolytic cleavage of many polysaccharides has been summarised. Since early work in this field has been adequately reviewed elsewhere, attention has been focused on the more recent developments, with particular reference to the use of enzymes in structural investigations of polysaccharides.

Enzymic studies have been used to determine the nature of the repeating unit in many polysaccharides (Table I), and have also been used to characterise the branching-linkages in a number of branched polysaccharides. In all cases, the results have confirmed those obtained by purely chemical methods. Furthermore enzymic studies provide the only available method for determining the degree of multiple branching in starch-type polysaccharides, and for locating the mean position of the branching points in the constituent chains.

It seems probable that during the next decade similar enzymic structural analyses of other polysaccharides will be possible as the appropriate polysaccharases are isolated, purified, and characterised. In the search for additional sources of polysaccharases, further study of the mammalian digestive systems and the enzyme systems of many higher plants would be of value. Perhaps the greatest promise in this field is in the adaptive production of polysaccharases by certain bacteria and moulds. Amylase, cellulase, dextranase, and polygalacturonase, essentially free from other polysaccharases, have been produced in this way. Although an extensive search may well be necessary to find a micro-organism which could be induced to grow in a medium containing, for example, araban as sole carbohydrate source, the specific arabanase(s) so produced would be an invaluable tool for the chemist engaged in structural investigations of the several plant gums and mucilages which contain L-arabinose.

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