Starch Biosynthesis

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1 Introduction

In the plant the ultimate source of energy is light, which, by photosynthesis, is used to manufacture carbohydrates, fats, and proteins, whereas the animal ingests these substances as food. Bulk reserve storage of energy is mainly achieved by the synthesis of starch in plants and of glycogen in animals. These polymers resemble one another quite closely, being based on the same monomeric unit, D-glucose. Their mode of synthesis also shows great similarities.

The starting point for the experimental investigation of the biosynthesis of polysaccharides was the discovery of the enzyme phosphorylase in 1937. This enzyme was found to catalyse the reversible incorporation of glucose 1-phosphate into glycogen. It was originally found in rabbit skeletal muscle[1] and, shortly afterwards, similar enzymic activity was found in a variety of plant materials which synthesised starch instead of glycogen.[2] Various other enzymes involved in polysaccharide biosynthesis were subsequently isolated and characterised but there still remains some doubt as to whether all the enzymes have been isolated.[3,4]

Theories of biosynthesis have mainly been concerned with the production of starch, since there are fewer unsolved problems relating to this enzyme system than that synthesising glycogen.[5] This article will therefore be confined to starch biosynthesis but some of the strong parallels between the two systems will be noted.[6]

Starch and its Components.—In plant sources starch occurs in the form of discrete granules. The starch granules are normally found in colourless plastids (leucoplasts) within the plant cell. When the leucoplasts are specialised as starch-storing bodies they are referred to as amyloplasts. One or more starch granules may arise in the amyloplast. The plastids involved in photosynthesis (chloroplasts) also commonly synthesise 'assimilation starch'.[7] However, this is a temporary product which remains in the plastid only as long as there is an excess of carbohydrates within the cell.

[1] G. T. Cori, S. P. Colowick, and C. F. Cori, *J. Biol. Chem.*, 1938, **123**, 375; H. Süllmann and R. Brückner, *Enzymologia*, 1940, **8**, 167.
[2] C. S. Hanes, *Nature*, 1940, **145**, 348; *Proc. Roy. Soc.*, 1940, *B*, **128**, 421; *ibid.*, 1940, *B*, **129**, 174.
[3] S. R. Erlander, *Enzymologia*, 1958, **19**, 273.
[4] R. B. Frydman and C. E. Cardini, *Arch. Biochem. Biophys.*, 1966, **116**, 9.
[5] W. J. Whelan, 'Lectures on the Scientific Basis of Medicine', The Athlone Press, London, 1956—1957, VI, p. 233.
[6] V. N. Nigam, *Arch. Biochem. Biophys.*, 1967, **120**, 214.
[7] L. W. Sharp, 'Introduction to Cytology', 3rd edn. McGraw-Hill, New York, 1934.

Starch is composed of two polymers amylose and amylopectin, both based on the same monomer, α-D-glucopyranose. The two polymers were first quantitatively separated from their heterogeneous mixture within the granule in 1942;[8] other, gentler, methods of isolation have since been developed.[9]

Most native starches have been found to contain *ca.* 20% amylose. This is an essentially linear polymer in which the glucose units are linked by (1 → 4)-α-glucosidic bonds. There are reports indicating a slight amount of branching in the molecule which is shown by incomplete β-amylolysis (see below).[10] Molecular weights are generally about 10^5—10^6.

The major component of starch, amylopectin, has the same (1 → 4)-α-linked backbone as amylose but this is interspersed with (1 → 6)-α-glucosidic bonds (*ca.* 1 in 20—30 monomer units) conferring on it a branched structure. Its molecular weights are generally about 10^7—10^8. Various theoretical structures for amylopectin have been proposed and the most conclusive evidence to date, based on enzymic degradation studies,[11] suggests that the 'ramified tree structure', a randomly branched model (Figure 1), is the most likely.[12]

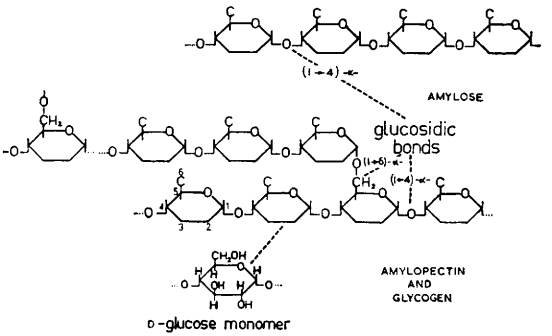

Fig. 1 *Basic structures of amylose, amylopectin, and glycogen.*

The chemistry of amylose and amylopectin has been extensively reviewed[13] and only some specific, potentially relevant, properties will be discussed here.

Amylose has a pronounced tendency to precipitate spontaneously out of aqueous solution, a phenomenon referred to as retrogradation. This behaviour is complex and dependent upon a number of factors including pH, concentration, and the molecular size of the amylose.[14] Retrogradation is imperfectly understood but is apparently due to associative forces causing parallel aggregation of

[8] K. H. Meyer, *Tech. Ind. Schweiz. Chem.-Ztg.*, 1942, **25**, 37.
[9] C. T. Greenwood, *Stärke*, 1960, **12**, 169.
[10] B. N. Stepanenko and E. M. Afanas'eva, *Biochem.* (*U.S.S.R.*), 1957, **22**, 285; R. Geddes, C. T. Greenwood, A. W. MacGregor, A. R. Proctor, and J. Thomson, *Makromol., Chem.*, 1964, **79**, 189.
[11] J. Larner, B. Illingworth, C. F. Cori, and G. T. Cori, *J. Biol. Chem.*, 1952, **119**, 641.
[12] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, 1940, **23**, 875.
[13] C. T. Greenwood, *Adv. Carbohydrate Chem.*, 1956, **11**, 335.
[14] J. F. Foster and M. D. Sterman, *J. Polymer Sci.*, 1950, **5**, 745; F. A. Loewus and D. R. Briggs, *J. Amer. Chem. Soc.*, 1957, **79**, 1494; M. Ceh and N. Vene, *Stärke*, 1959, **11**, 290.

the long linear amylose chains until colloidal dimensions are exceeded and precipitation occurs.[8]

Amylose gives a deep blue stain with iodine solution while amylopectins give stains of various shades of violet. This can be used as a useful diagnostic *in vivo* test for amylose. The phenomenon is generally presumed to be due to resonance of complex iodide ions within the amylose molecule in its helical conformation. Whether the conformation of the amylose in solution is, in fact, basically helical, or whether the helix is an artefact of the addition of iodine is not known. Two recent (1963) studies on the conformation of amylose in aqueous solutions failed to agree fundamentally, and the problem is still unresolved.[15]

Appreciable amounts of phosphorus (up to 0·2%) are generally found to be associated with starches. It has been shown that most of the phosphorus in cereal starches is in the form of phosphatides which can be extracted by hot water or methanol.[16] On the other hand the phosphorus in tuber starches, such as potato, sago, tapioca, *etc.* was found to be linked to the polysaccharides in ester form through the C-6 position. About 90% of this phosphorus has been shown to be linked to the amylopectin molecules.[17] However the action of the enzyme phosphatase upon this esterified phosphorus in potato starch is apparently hindered by the interaction of neighbouring glucosyl chains with the phosphate groups.[18]

There has been a suggestion that the very high molecular weights of amylopectins may arise from aggregates of smaller molecules,[19] a phenomenon common among proteins.[20] However, unlike the proteins, both amylose and amylopectin are very polydisperse.[21]

2 Experimental Approaches to the Study of Starch Biosynthesis

The study of the synthesis of starch within the granule has generally been approached from three broad aspects: (A) investigation of the physical nature of the starch granule itself by light- and electron-microscopy and of the effect of chemical and physical agents upon the structure of the granule, (B) isolation and characterisation of the enzymes responsible for starch biosynthesis, and (C) characterisation of the products of starch biosynthesis and their variation during the growth of the plant. Most investigations can be classified under one of these headings. However some recent work has combined a genetic approach, the investigation of mutants, and the investigation of the enzymes present, comparing both aspects with the normal plant.[22] This is a potentially powerful approach which could allow some insight into the genic control of starch biosynthesis.

[15] W. Banks and C. T. Greenwood, *Makromol. Chem.*, 1963, **67**, 49; V. S. R. Rao and J. F. Foster, *Biopolymers*, 1963, **1**, 527.
[16] T. Posternak, *Helv. Chim. Acta*, 1935, **18**, 1351.
[17] T. J. Schoch, *J. Amer. Chem. Soc.*, 1942, **64**, 2954, 2957.
[18] T. Fukui, *Mem. Inst. Sci. Ind. Res., Osaka Univ.*, 1958, **15**, 201.
[19] S. R. Erlander and D. French, *J. Polymer Sci.*, 1958, **32**, 291.
[20] I. M. Klotz, *Science*, 1967, **155**, 697.
[21] C. J. Stacey and J. F. Foster, *J. Polymer Sci.*, 1957, **20**, 67.
[22] C. Y. Tsai and O. E. Nelson, *Science*, 1966, **151**, 341.

Previous genetic studies had mainly been concerned with the effect of different gene combinations on the chemical composition of starches.[23]

A. Physical Nature of the Starch Granule.—The starch granules which arise in the plastids of different species of plant cells show such extensive morphologic variation that they may be used for the identification of seeds and other starch-containing plant parts. Typical size variations are: potato 70—100 μ, wheat 30—40 μ, maize 10—20 μ.[24,25] When starch granules are immersed in water they swell and then become transparent. Simultaneously a characteristic layered appearance becomes visible under the optical microscope. The granules also exhibit birefringence under crossed Nicols.[24] The layers indicate that the granules are formed of shells composed of material with differing optical characteristics. They are usually alternately crystalline (birefringent) and amorphous. Layering is less obvious in the cereal than in other starches but it can be emphasised by treatment of the granules with dilute acid.

The formation of the layers was originally thought by Meyer in 1895 to arise from diurnal variations in the biosynthesis of the starch.[26] This has been conclusively disproved for potato starch granules.[27] However, in the case of corn starch, it has been found that amylose and amylopectin are produced at the same time of the day but that the production of these components is increased during the night.[28] Buttrose[29] has compared the formation of layers in the cases of wheat and potatoes. He has found that potato starch granules grown in a constant environment of light and temperature have the same shell structure as granules from normal potatoes. On the other hand wheat starch granules grown under the same conditions have no shell structure but this can be produced at will by imposing a dark period. He concludes that shell formation is controlled by an endogenous mechanism in potatoes whereas in wheat it is controlled by the environment rather in the manner envisaged by Meyer.

The granules normally contain *ca.* 75% amylopectin although there can be large variations among species (*e.g.* 'waxy' cereal starches are composed of effectively 100% amylopectin). It is thought that the parallel arrangement of the outer branches of amylopectin is responsible for the pronounced birefringence of the starch granules.[24] This is substantiated by the observation that starch from waxy cereals has the same intensity of birefringence as that from normal types.

The suggestion[30] that the layers might be alternately composed of amylose and amylopectin has been disproved;[28,31] there is no chemical distinction between the layers and the linear molecules are evenly distributed throughout the

[23] J. W. Cameron, *Genetics*, 1947, **32**, 459; R. L. Whistler, H. H. Kramer, and R. D. Smith, *Arch. Biochem. Biophys.*, 1957, **66**, 374.
[24] N. P. Badenhuizen, *Protoplasmatologia*, 1959, IIB2bδ.
[25] K. Esau, 'Plant Anatomy', 2nd edn., Wiley, New York, 1965.
[26] A. Meyer, 'Untersuchungen über die Stärkenkörner', Fischer, Jena, 1895.
[27] E. Bünning and C. Hess, *Naturwiss.*, 1954, **41**, 339; E. A. Roberts and B. E. Proctor, *Science*, 1954, **119**, 509; M. G. Mes and I. Menge, *Physiol. Plantarum*, 1954, **7**, 637.
[28] S. R. Erlander, *Cereal Chem.*, 1960, **37**, 81.
[29] M. S. Buttrose, *Stärke*, 1963, **15**, 213.
[30] A. T. Czaja, *Planta*, 1954, **43**, 379.
[31] J. M. G. Cowie and C. T. Greenwood, *J. Chem. Soc.*, 1957, 2862.

layers of the granule.[32] Very fine layering has been shown by electron-microscopy.[33]

Apposition. The mode of production of the starch granule was in question for a considerable time. Two possibilities were (i) chemical coacervation, *i.e.* retrogradation of the synthesised mixture of amylose and amylopectin and (ii) apposition, *i.e.* growth of the granule by successive depositions of synthesised material on the outside of the granule. Apposition seems to be the more likely, especially since it appears to offer some explanation for the layered appearance of the granules. However the suggested mechanism of coacervation shows similarity to the retrogradation of amylose in aqueous solutions.[14] It was not until 1956 that the use of ^{14}C labelling of substrate material and subsequent autoradiographs of the granules confirmed apposition as the method of deposition of the granules.[34] This was further substantiated by later workers.[35] (A recent examination of various types of starch granules by electron-microscopy has demonstrated the presence, within the granular layers, of elementary grains with diameters between 150 and 250 Å. Further, the presence of a membrane is noted, which the authors suggest is composed of amylose. However they freely admit that this hypothesis is 'undoubtedly daring'.)[36]

B. Enzymes Involved in Starch Biosynthesis.—The first enzyme to be isolated which was involved in starch biosynthesis was phosphorylase (*P*-enzyme).* It was isolated by Hanes in 1940, from potato juice.[2] This enzyme was found to catalyse the reversible synthesis of the (1 → 4)-α-glucosidic bond and with the discovery, in 1944, of *Q*-enzyme, which synthesises the (1 → 6)-α-glucosidic bond,[37] it appeared as if the solution to the problem of starch biosynthesis might be unusually simple. However, subsequent investigations have indicated the presence of further enzymes in plant sources which must be involved in the biosynthesis: *R*-enzyme (1951),[38] *D*-enzyme (1956),[39] 'amylose synthetase' (1957),[40] *T*-enzyme (1960),[41] and ADP-glucose pyrophosphorylase (1962).[42] In addition to these enzymes the degradative α- and β-amylases, which catalyse the hydrolysis of (1 → 4)-α-glucosidic bonds, are found in plant sources.[24]

Concurrently with these discoveries a series of similar enzymes apparently involved in the production of glycogen was isolated from both animal and micro-organism sources. Recently also, the biosynthesis of starch in green algae has

*The terms 'phosphorylase' and *P*-enzyme are here treated as being synonymous. See, however, D. J. Manners, *Adv. Carbohydrate Chem.* 1962, **17**, 418.

[32] N. P. Badenhuizen, *Cereal Chem.*, 1955, **32**, 286.
[33] M. S. Buttrose, *J. Cell. Biol.*, 1962, **14**, 159.
[34] N. P. Badenhuizen and R. W. Dutton, *Protoplasmatologia*, 1956, **47**, 156.
[35] M. Yoshida, M. Fujii, Z. Nikuni, and B. Maruo, *Bull. Agr. Chem. Soc. Japan*, 1958, **21**, 127.
[36] J. B. Donnet, L. Medemblik, and J. Jaeger-Messiet, *Bull. Soc. chim. France*, 1967, 2117.
[37] W. N. Haworth, S. Peat, and E. J. Bourne, *Nature*, 1944, **154**, 236.
[38] P. N. Hobson, W. J. Whelan, and S. Peat, *J. Chem. Soc.*, 1951, 1451.
[39] S. Peat, W. J. Whelan, and G. W. F. Kroll, *J. Chem. Soc.*, 1956, 53.
[40] L. F. Leloir and C. E. Cardini, *J. Amer. Chem. Soc.*, 1957, **79**, 6340.
[41] M. Abdullah and W. J. Whelan, *Biochem. J.*, 1960, **75**, 12P.
[42] J. Espada, *J. Biol. Chem.*, 1962, **237**, 3577.

been shown to proceed in a manner similar to that in plants.[43] In this latter case in particular, the 'amylose synthetase' enzyme (isolated from *Chlorella pyrenoidosa*) was found to be similar to the corresponding enzyme derived from the leaves of the higher plants as well as that found in bacterial extracts. In general, the glycogen-synthesising and -degrading enzymes of animals correspond in type to those of plants but differ from them in many points of detail.[5]

The nature of the enzymes isolated from plant sources will now be discussed. (i) *Phosphorylase.* Phosphorylase was originally[1] found in skeletal muscle and was subsequently demonstrated to be present in liver, heart, brain, and other animal tissues.[44] The enzyme was also shown to be present in yeast and then, by Hanes,[2] in peas, potatoes, and other plant materials. These early investigations showed that the reaction catalysed by phosphorylase was freely reversible in the following manner:

Glucose 1-phosphate + $[\text{glucose}]_n \rightleftharpoons [\text{glucose}]_{n+1}$ + inorganic phosphate

The position of the equilibrium is not affected by the concentration of the oligo- or poly-saccharide $[\text{glucose}]_n$ within quite wide limits and therefore depends on the ratio of glucose 1-phosphate to inorganic phosphate.[45] This equilibrium ratio has been measured by Hanes[2] and is shown in the Table. The

Equilibrium ratios of glucose 1-phosphate (G) to inorganic phosphate (P) for phosphorylase catalysis

pH	5	6	7
G : P	10·8	6·7	3·1

dependence of the nature (*i.e.* synthetic or degradative) of phosphorylase activity upon the concentration of inorganic phosphate is clearly emphasised. As inorganic phosphate accumulates, the synthetic reaction will slow down. With an excess of inorganic phosphate and without primer (see below) amylose is theoretically hydrolysed to glucose. In fact only 70% of the amylose is normally converted, in a similar manner to the lack of complete hydrolysis with β-amylase.[10] Amylopectin forms a phosphorylase limit dextrin when approximately half of the molecule has been consumed.

However by careful regulation of this equilibrium synthetic amylose has been prepared with degrees of polymerisation (D.P.) as great as 12,500.[46] Differences in behaviour between the natural and synthetic amylose were concluded to be due to the heterogeneity of the former.

The presence of a primer molecule was found to be necessary for efficient synthesis. For potato phosphorylase a satisfactory primer must be composed of at least three glucose units linked by (1 → 4)-α-glucosidic bonds (maltotriose).[47] However the priming activity of maltotriose is only 9% of that of maltotetrose.[48]

[43] J. Preiss and E. Greenberg, *Arch. Biochem. Biophys.*, 1967, **118**, 702.
[44] C. T. Cori, S. P. Colowick, and C. F. Cori, *J. Biol. Chem.*, 1938, **123**, 375.
[45] G. R. Cori and C. F. Cori, *J. Biol. Chem.*, 1940, **135**, 733.
[46] E. Husemann, W. Burchard, and B. Pfannemüller, *Stärke*, 1964, **16**, 143.
[47] D. French and G. M. Wild, *J. Amer. Chem. Soc.*, 1953, **75**, 4490.
[48] W. J. Whelan and J. M. Bailey, *Biochem. J.*, 1954, **58**, 560.

The function of the primer is to provide non-reducing terminal units as foundations for the chain-lengthening process. Synthesis by *P*-enzyme was originally thought to occur by multi-chain action[48] (*i.e.* any particular enzyme molecule adds monomer randomly to any of the growing chains, in contrast to single-chain action where the enzyme molecule extends one particular polymer chain exclusively), but later work has thrown some doubt on this conclusion.[49] The possibility that the polymerisation proceeds by a single-chain mechanism cannot be eliminated.

A novel regulatory mechanism for the action of phosphorylase on starch granules has been shown to exist. Adsorption of phosphorylase on starch granules was found to depend on the composition of the surrounding medium. It was suggested that a similar mechanism operates in the case of 'amylose synthetase'.[50]

(ii) Q-*Enzyme*. *Q*-Enzyme was originally isolated from potatoes and although it is present in other plant sources, the potato has been its principal source. *Q*-Enzyme catalyses a glucosidic exchange reaction in which a (1 → 4)-α- is converted into a (1 → 6)-α-glucosidic bond.[51] The enzyme splits a (1 → 4)-α-link in a donor molecule of amylose and attaches the fragment removed through a (1 → 6)-α-bond. Whether the same molecule can act both as donor and acceptor is not known. To be an acceptor the amylose must have a degree of polymerisation greater than 42 (ref. 52). *Q*-Enzyme can also act on the outer branches of amylopectin which consist of at least 14 glucose units.[53] However *Q*-enzyme cannot convert amylopectin into the much more highly branched glycogen.[54]

Q-Enzyme exerts a marked accelerating effect upon the rate of polysaccharide synthesis by *P*-enzyme.[55] This autocatalytic effect is presumably caused by the increasing numbers of non-reducing end-groups produced by the action of *Q*-enzyme.

(iii) R-*Enzyme*. *R*-Enzyme, like β-amylase, has been isolated from the higher plants. It operates in the reverse manner to *Q*-enzyme, hydrolysing the (1 → 6)-α-inter-chain bonds. It only hydrolyses a proportion of the bonds in amylopectin and has no effect on glycogen,[56] probably owing to steric hindrance.

(iv) D-*Enzyme*. In the linear synthesis of oligosaccharides and amylose, catalysed by phosphorylase and 'amylose synthetase' enzymes, an acceptor molecule (primer) must consist of at least four glucose units to work efficiently. The function of *D*-enzyme is thought to be to provide these molecules from smaller molecules by a disproportionation reaction of the following type:[39,57]

2 maltotriose ⇌ glucose + maltopentose

D-Enzyme is present in appreciable amounts in potato juice. It has no action on

[48] E. E. Smith and W. J. Whelan, *Biochem. J.*, 1963, **88**, 50P.
[50] M. A. R. De Fekete, *Arch. Biochem. Biophys.*, 1966, **116**, 368.
[51] S. A. Barker, E. J. Bourne, and S. Peat, *J. Chem. Soc.*, 1949, 1712.
[53] S. Nussenbaum and W. Z. Hassid, *J. Biol. Chem.*, 1952, **196**, 785.
[54] J. Larner, *J. Biol. Chem.*, 1953, **202**, 491.
[55] D. J. Manners, *Adv. Carbohydrate Chem.*, 1962, **17**, 371.
[56] H. B. N. Murthy, G. R. Rao, and M. Swaminathan, *Enzymologia*, 1957, **18**, 634.
[57] S. Peat, W. J. Whelan, P. N. Hobson, and G. J. Thomas, *J. Chem. Soc.*, 1954, 4440.
[57] S. Peat, W. J. Whelan, and W. R. Rees, *J. Chem. Soc.*, 1956, 44.

glucose or maltose but with large maltodextrins it functions as indicated above. Some of the oligosaccharides synthesised are long enough to cause staining with iodine. The rate of synthesis of this iodine-staining material is more than doubled when hexokinase and ATP are added.[58]

(v) T-*Enzyme*. *T*-Enzyme can be isolated from the juice of potatoes. It appears to be complementary to *Q*-enzyme in that it introduces branching into short chain oligosaccharides, which *Q*-enzyme will not. A typical reaction is:

$$2 \text{ maltose} \rightarrow \text{panose} + \text{glucose}$$

The reaction is irreversible.

(vi) *Amylose Synthetase*. The discovery of 'amylose synthetase' in plant sources probably constitutes the most significant recent advance in the investigation of starch biosynthesis. 'Amylose synthetase' is a general term applied to the enzyme which catalyses the incorporation of a glucose unit into amylose, amylopectin, or low molecular weight oligomers of these molecules. The starting materials for these enzymes are the nucleotide sugars, ADP–glucose and UDP–glucose, and a more precise description of the enzyme is ADP–glucose : starch transglucosylase or UDP–glucose : starch transglucosylase. When the action of this enzyme is coupled with that of a pyrophosphorylase (see below) a simple reaction scheme starting with glucose 1-phosphate (Figure 2) can be visualised. An enzyme

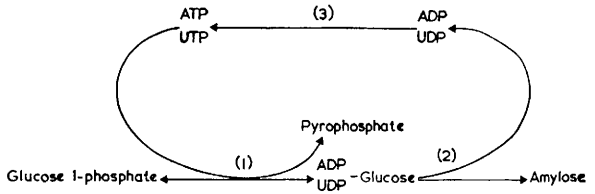

Fig. 2 *Pathway for the incorporation of glucose* 1*-phosphate into amylose* via *nucleotide sugars.*

system (3) in the Figure which accomplishes the change of ADP (UDP) into ATP (UTP) is located in mitochondria, but since it is difficult to distinguish young plastids from mitochondria the oxidative phosphorylation may be accomplished within the amyloplast.

A number of sugar nucleotides can act as glucosyl donors[59] but ADP–glucose and possibly UDP–glucose appear to be the natural substrates for this enzyme. Leloir considers that the 'amylose synthetase' pathway probably consists of two parallel paths with ADP–glucose or UDP–glucose as substrates.[60] However De Fekete and Cardini have postulated that the primary rôle of UDP–glucose may be in the initial step of the sucrose–starch transformation.[61] The mechanism which they suggested for this latter transformation has been criticised.[62]

[58] G. J. Walker and W. J. Whelan, *Nature*, 1959, **183**, 46.
[59] R. B. Frydman and C. E. Cardini, *Biochim. Biophys. Acta*, 1965, **96**, 294.
[60] L. F. Leloir, *Biochem. J.*, 1964, **91**, 1.
[61] M. A. R. De Fekete and C. E. Cardini, *Arch. Biochem. Biophys.*, 1964, **104**, 173.
[62] T. Murata, T. Sugiyama, T. Minamikawa, and T. Akazawa, *Arch. Biochem. Biophys.*, 1966, **113**, 34.

'Amylose synthetase' was found to be bound to the surface of the starch granule;[63] however, the enzymic properties vary to some extent with the source. The difficulty of studying primer specificity and action-pattern while the enzyme is still bound to the starch granule led to a search for a soluble form. The soluble enzyme has now been obtained from several sources, including potato tubers.[64] The soluble 'amylose synthetase' is adsorbed to amylose, retrograded amylose of low molecular weight, and to 'particulate' glycogen.[64] The adsorbed amylopectin can use these polysaccharides as glucosyl acceptors.

In the dark, green plants lose both their starch and the 'amylose synthetase' activity in their juice. The latter returns after re-exposure to light.[65]

(vii) *ADP-Glucose pyrophosphorylase.* This enzyme has only been isolated from wheat extracts,[42] spinach leaf chloroplasts,[66] and rice granules,[67] but the importance of nucleotide sugars to polysaccharide synthesis makes it possible that it is more widely distributed in plant sources. (However, in the case of potato tubers, it has been suggested that the formation of ADP-glucose *in vivo* must proceed through sucrose synthetase since ADP-glucose pyrophosphorylase has not been detected in the tubers.[42]) It catalyses the reaction:

$$\text{glucose 1-phosphate} + \text{ATP} \rightleftharpoons \text{ADP-glucose} + \text{pyrophosphate}$$

Starch synthesis in the spinach leaf appears to be regulated at the level of ADP-glucose and hence is controlled by this enzyme.[66] This is obviously a fruitful field for future research.

C. Studies of the Products of Biosynthesis.—The study of the products of biosynthesis might well be called the macroscopic approach to the problem of starch biosynthesis. This has probably been the least useful but nevertheless a widely studied aspect of biosynthesis. There appear to be two main reasons for this: firstly, it is of economic importance to know under what conditions the maximum starch yield occurs and the effect of the ratio of amylose to amylopectin in determining the properties and preparation of starch products. Secondly, the study is relatively simple experimentally, and consequently the range of species which has been studied by this method is wide, *e.g.* potato tubers,[68,69] barley,[70] and peas.[71]

In all cases it was shown that as the plant matures, the starch-content, the granule-size, and the ratio of amylose to amylopectin all increase. A more detailed physico-chemical investigation[69] of the separated products indicates that

[63] L. F. Leloir, M. A. R. De Fekete, and C. E. Cardini, *J. Biol. Chem.*, 1961, **236**, 636.
[64] R. B. Frydman and C. E. Cardini, *Arch. Biochem. Biophys.*, 1966, **116**, 9.
[65] K. R. Chandorkar and N. P. Badenhuizen, *Cereal Chem.*, 1967, **44**, 27.
[66] H. P. Ghosh and J. Preiss, *J. Biol. Chem.*, 1966, **241**, 4491.
[67] Y. Tanaka, S. Minagawa, and T. Akazawa, *Stärke*, 1967, **19**, 206.
[68] A. N. Petrova and T. T. Bolotina, *Biochem.* (*U.S.S.R.*), 1956, **21**, 457.
[69] R. Geddes, C. T. Greenwood, and S. MacKenzie, *Carbohydrate Res.*, 1965, **1**, 71.
[70] G. Harris and I. C. MacWilliam, *Cereal Chem.*, 1958, **35**, 82.
L. H. May and M. S. Buttrose, *Austral. J. Biol. Sci.*, 1959, **12**, 146.
[71] C. T. Greenwood and J. Thomson, *Biochem. J.*, 1962, **82**, 156.

as the plant matures the molecular size of both the amylose and amylopectin components increases and there is a corresponding decrease in the extent of conversion of these components into maltose by β-amylase. The presence of a 'third component' with properties intermediate between amylose and amylopectin has been reported in some cases.[72,73] Also, as previously noted, a slight degree of branching in amylose has been detected.[10]

McConnell and his co-workers[72] used a variation of this general approach in their biosynthetic studies. They isolated and fractionated starches from kernels of wheat which had been administered [1 — ^{14}C] glucose, [1 — ^{14}C] acetate, or [2 — ^{14}C] acetate. (Acetate is probably an intermediate in the pathway for the conversion of fatty acids into carbohydrates. Evidence for such a pathway has been found in many plants.[74]) They found notable differences in the specific activity of the starch fractions (including the 'third component'), and in the distribution of ^{14}C within the monomeric units of samples labelled by means of [1 — ^{14}C] glucose. The results were interpreted as indicating that the process of starch deposition was not wholly reversible and also that amylopectin was formed from amylose. The minor 'third component' was possibly an intermediate in this latter conversion.

3 The Theories of Starch Biosynthesis

It appears that the enzymes described above could provide a simple pathway for the biosynthesis of starch from glucose 1-phosphate. Amylose can be formed by the synthetic action of phosphorylase and this has been demonstrated *in vitro*.[46] Alternatively, or in addition, the ADP- and UDP-glucose : starch transglucosylase can synthesise amylose. Amylopectin is formed by the action of *Q*- and *T*-enzymes upon linear substrates provided by one, or more, of these 'linear pathways'.

Unfortunately the concurrent synthesis of amylose and amylopectin in the starch granule cannot be explained in this manner, since mixtures of enzymes which catalyse the synthesis of (1 → 4)-α- and (1 → 6)-α-glucosidic bonds inevitably give branched material, albeit with various degrees of branching, dependent upon the ratios of the various enzymes. This has been clearly demonstrated *in vitro*.[75] The main problem, therefore, in theorising on the mode of starch biosynthesis is the manner in which enzymic activity can be restricted.

The current theories of starch biosynthesis have been suggested by Whelan (1958 and 1963),[76,77] Erlander,[3] and Geddes and Greenwood (1968).[78] None of these theories has been verified experimentally. Their merits and shortcomings will now be discussed.

[72] W. B. McConnell, A. K. Mitra, and A. S. Perlin, *Canad. J. Biochem. Physiol.*, 1958, **36**, 985.
[73] A. S. Perlin, *Canad. J. Chem.*, 1958, **36**, 810; W. Banks and C. T. Greenwood, *J. Chem. Soc.*, 1959, 3436.
[74] H. L. Kornberg and H. Beevers, *Nature*, 1957, **180**, 35; M. T. Heydeman, *Nature*, 1958, **181**, 627; C. Bradbeer, *ibid.*, 1958, **182**, 1429.
[75] S. A. Barker, E. J. Bourne, S. Peat, and I. A. Wilkinson, *J. Chem. Soc.*, 1950, 3022.
[76] W. J. Whelan, *Handbuch der Pflanzenphysiologie*, 1958, **6**, 154.
[77] W. J. Whelan, *Stärke*, 1963, **15**, 247.
[78] R. Geddes and C. T. Greenwood, *Stärke*, in the press.

(i) *Whelan* (1958) *theory*. This theory is illustrated in Figure 3. It postulates the presence in the synthesising system of a membrane which is permeable to D-glucose and maltosaccharides of D.P. $\leqslant 4$ only. The proposal of this theory predates the isolation of ADP- and UDP-glucose : starch transglucosylase in plant sources. However the addition of these enzymes would not disturb the basic idea behind this theory. The fundamental postulate in this case is the separation, by the membrane, of the amylose and amylopectin synthesis, *Q*-enzyme being only able to act on material which has diffused through the membrane.

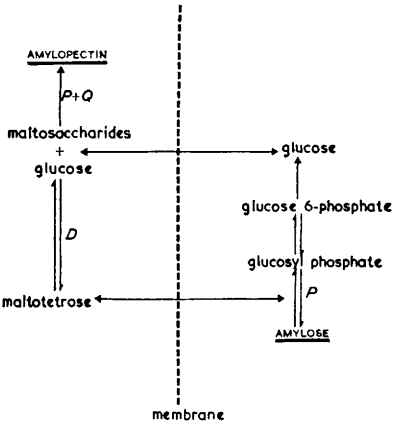

Fig. 3 *Scheme of the Whelan* (1958) *theory.*

The main objection to this theory is the invocation of a semi-permeable membrane with very specific properties. Further, this membrane would cause an actual physical separation of the synthesised amylose and amylopectin within the granule, which seems unlikely in view of the accumulated chemical evidence on the nature of the granule.

However, electron-microscopic examination of starch granules has provided some evidence for membranes, [36,79] so that the postulate cannot be entirely ignored. Further, amylose can be removed preferentially from the granule by either 'chemical' means, *e.g.* with sodium salicylate[80] or urea,[64] or by gentle leaching.[31] Removal of the amylose suggests that the amylopectin itself, might act as a membrane.

(ii) *Whelan* (1963) *theory*. This idea of a membrane composed of amylopectin was invoked in the later theory of Whelan, which is illustrated in Figure 4. The theory utilises the ADP- and UDP-glucose : starch transglucosylase, the significance of

[79] A. Guilbot and G. Levavasseur, Proceedings of 3rd International Conference on Electron Microscopy, London, 1954, p. 533.
[80] M. Ulmann, *Stärke*, 1956, **8**, 109.

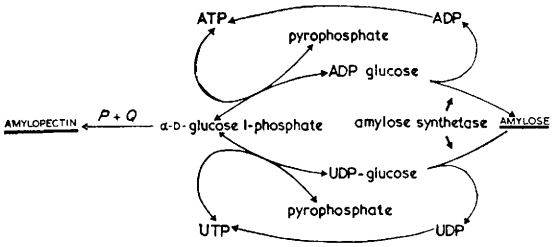

Fig. 4 *Scheme of the Whelan* (1963) *theory.*

which was fully appreciated only after the appearance of the 1958 theory. A fundamental change embodied in the new theory was that the synthesis of amylose and amylopectin were assumed not to be so closely linked as was normally presumed. In favour of this suggestion the observation[81] was cited that normal maize granules contain UDP–glucose : starch transglucosylase activity but waxy maize granules do not. Since the waxy maize granules contain 100% amylopectin this observation implied that amylopectin was synthesised by a mechanism other than the 'amylose synthetase' pathway. In Figure 4 it can be seen that amylopectin is synthesised by the concurrent action of *P*- and *Q*-enzymes, while amylose is formed totally independently by the ADP- and UDP–glucose : starch transglucosylase pathways. The action of *Q*-enzyme upon the products of the latter pathway is limited by the proposed semi-permeable effect of the amylopectin. Whelan[77] suggests that 'if the enzyme that synthesises amylose becomes surrounded by amylopectin then *Q*-enzyme would not be able to penetrate to the amylose, whereas the sugar nucleotide substrate, a much smaller molecule, could pass through.'

This theory was partially invalidated in the same year that it was published. Frydman[82] discovered ADP–glucose : starch transglucosylase activity in sweet corn (100% amylopectin). This obviously provides an alternative, very similar, pathway to that which would have been provided by the UDP–glucose : starch transglucosylase activity which was absent in waxy maize. Indeed there are indications that ADP–glucose rather than UDP–glucose is the natural substrate for the enzyme.[83] This observation, which shows that the synthetic pathways to amylose and amylopectin are not separate, is further substantiated by the evidence that amylose (or amylose-like dextrins) is present in waxy maize five to seven days after pollination.[84] It can be concluded therefore that waxy maize, under normal conditions, effects complete conversion of amylose into amylopectin, whereas with the normal varieties the process is much less efficient.

(iii) *Erlander* (1958) *theory.* A unique approach to the problem of starch biosynthesis was suggested by Erlander in 1958.[3] He postulated that starch was

[81] O. E. Nelson and H. W. Rines, *Biochem. Biophys. Res. Comm.*, 1962, **9**, 297.
[82] R. B. Frydman, *Arch. Biochem. Biophys.*, 1963, **102**, 242.
[83] T. Murata, T. Minamikawa, and T. Akazawa, *Biochem. Biophys. Res. Comm.*, 1963, **13**, 439; T. Murata and T. Akazawa, *ibid.*, 1964, **16**, 6.
[84] H. Fuwa, *Nature*, 1960, **179**, 159; H. Fuwa, *Arch. Biochem. Biophys.*, 1957, **70**, 157.

synthesised from glycogen in the plant by a debranching enzyme, which had yet to be isolated. That glycogen should be an intermediate in the formation of starch was suggested by the presence of a glucogen-type polysaccharide in sweet-corn endosperm.[85] This stimulated Erlander to question how the spherical, highly-branched glycogen, the asymmetric, less closely branched amylopectin, and the linear amylose could all be produced in the same vicinity. He therefore proposed the synthetic pathway shown in Figure 5.

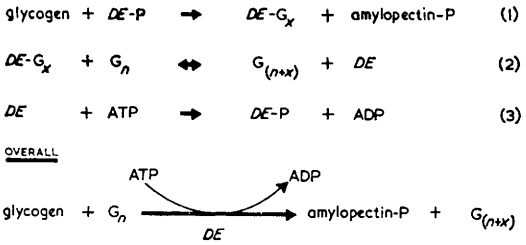

Fig. 5 *Scheme of the Erlander* (1958) *theory.*

The phosphorylated debranching enzyme (*DE*–P) transfers its phosphate group (P) to the branch point of the glycogen molecule and simultaneously becomes linked with the aldehydic group of the branch. The complex (*DE*–G_x) between the debranching enzyme (*DE*) and the debranched chain (G_x) containing x glucose units would be similar to that enzyme complex involved in the branching of glycogen.[86] The *DE*–G_x complex can then transfer its chain to the non-reducing end of the receptor group (G_n). This theoretical debranching enzyme, after being phosphorylated by some agent such as ATP, can again attack another glycogen molecule. The resulting linear G_{n+x} molecule, now having $(n + x)$ glucose units, can again act as an acceptor. In this manner the long linear chains of amylose can be rapidly formed, enabling the amylose to crystallise out of the medium before the branching enzyme can attack it.

There are several reasons why this theory has received criticism.[54] Firstly, a debranching enzyme such as *DE* has not been detected in any plant source. *R*-enzyme (see above) has no action whatsoever on glycogen. This is reasonable when it is considered how compact the interior of a glycogen molecule is: the average internal chain-length is only three or four glucose units. Even the randomly acting α-amylases cannot penetrate the centre of the glycogen molecule.[87] However, the presence of an enzymic system in corn endosperm which acts on amylose and amylopectin to give a glycogen has been reported.[88] Evidence has also been presented for the presence in sweet corn of an enzyme which can introduce branches into amylopectin.[89] Further, a glycogen-debranching

[85] W. Z. Hassid and R. M. McCready, *J. Amer. Chem. Soc.*, 1941, **63**, 1632.
[86] S. A. Barker and E. J. Bourne, *Quart. Rev.*, 1953, **7**, 56.
[87] R. Geddes, *Carbohydrate Res.*, 1968, **7**, 493.
[88] N. Lavintman and C. R. Krisman, *Biochim. Biophys. Acta*, 1964, **89**, 193.
[89] D. J. Manners and J. J. M. Rowe, *Chem. and Ind.*, 1964, 1834.

system has recently been discovered in yeast.[90] Therefore there is still a possibility that an enzyme such as *DE* could exist undetected as yet, in plant sources.

A second criticism of the theory is that only in sweet corn has a glycogen-type polysaccharide been isolated. However if reaction (1) in Figure 5 was very rapid then it might be necessary to use more efficient extraction techniques. More particularly enzymic activity would have to be inhibited.

Nevertheless, on balance, it seems unlikely that glycogen is synthesised in plants, since none of the enzymes present are particularly suitable for the synthesis of glycogen. The 'amylose synthetase' and phosphorylase enzymes do not use glycogen as an efficient acceptor, although transfer of glucose does occur, and *Q*-enzyme is unable to introduce sufficient branch points.

The reader is referred to the detailed review by Manners[54] for a more extensive criticism of this theory and to the recent calculations by Erlander in support of the formation of a glycogen intermediate.[91]

(iv) *Geddes–Greenwood* (1968) *theory*. This theory (Figure 6) is similar in scheme

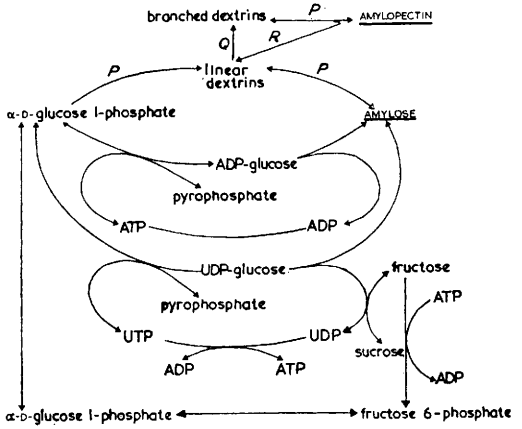

Fig. 6 *Scheme of the Geddes–Greenwood* (1968) *theory.*

to the Whelan (1963) theory. The pathway involving the utilisation of the fructose half of the sugar molecule is based on the work of Akazawa *et al.*,[92] who observed that in the presence of UDP–sucrose and UDP–glucose : starch transglucosylase there was a definite transfer of glucose from [^{14}C] sucrose to starch molecules. These workers also proposed the pathway linking fructose with glucose 1-phosphate shown in Figure 6.[92] The pathway involving the transfer of the 'sucrose glucose' is not illustrated for the sake of clarity.

Further evidence for the incorporation of this glucose into the starch molecules

[90] E. Y. C. Lee, L. D. Nielsen, and E. H. Fischer, *Arch. Biochem. Biophys.*, 1967, **121**, 245.
[91] S. R. Erlander, *Stärke*, 1967, **19**, 99.
[92] T. Akazawa, T. Minamikawa, and T. Murata, *Plant Physiol.*, 1964, **39**, 371.

has been provided by studies of corn endosperm. The enzymes required for either of the two transformations shown below were found to be present.[61]

sucrose ⇌ ADP– or UDP–glucose → starch
sucrose ⇌ UDP–glucose ⇌ glucose 1-phosphate ⇌ ADP–glucose starch

The essential postulate of this theory is the formation of a linear dextrin 'pool'. These dextrins (glucose oligomers) are formed by either of the transglucosylase pathways (the 'pool' on these pathways has again been omitted for the sake of clarity), by phosphorylase synthesis, or by degradation of amylose by phosphorylase. The 'amylose synthetase' enzyme is well known to be associated with the surface of the granule. It is reasonable therefore, that most of the synthesis of linear material would occur in the environment of the granule. In the lack of any specific evidence, *Q*-enzyme must be assumed to be freely distributed within the plastid. (If *Q*-enzyme were found to be associated with the plastid membrane it would be easy to conceive of the synthesis of both linear and branched material within the plastid. The author is unaware if any amyloplast or chloroplast membranes have been specifically tested for *Q*-enzyme activity. This would be a very significant experiment. It is known that many membranes within the cell are associated with enzymic activity.) There appear to be multiple sites for the binding of *Q*-enzyme and its substrate.[53] Further, *Q*-enzyme is more active towards branched than towards linear substrates.

It was postulated, therefore, that the intense synthesis of linear material at or near the starch granule would saturate *Q*-enzyme activity in the immediate vicinity and allow a proportion of the molecules to remain unbranched until their linear length is such that they precipitate out of solution. Chain extension of branched material may proceed by *P*-enzyme-catalysed synthesis or by an 'amylose synthetase' pathway. The concept of a linear dextrin 'pool' is very similar to the concept of 'pseudoamylose' put forward by Bourne and Peat in their original paper on *Q*-enzyme.[93]

This theory provides an explanation for the relative increase in amylose content, compared to amylopectin, which occurs with increase in granule size (and therefore with time of growth). As the surface area of the granule increases with size of the granule more 'amylose synthetase' will be absorbed from the interior of the amyloplast. This will cause more intensive production of linear material and hence a greater excess at the linear dextrin 'pool'. This excess will be the increase in amylose content.

This theory, like all the previous theories, emphasises our imperfect understanding of the mechanism of this relatively simple biosynthetic pathway. It is to be hoped that the postulates on which the various theories rely will be tested experimentally in the near future.

4 Conclusion

Our knowledge of the factors governing the biosynthesis of starch, the enzymes and substrates involved, and the physical and chemical nature of the products, is

[93] E. J. Bourne and S. Peat, *J. Chem. Soc.*, 1945, 877.

constantly increasing and still the theories proposed have to rely on speculations. This may be because an individual research group approaches the problem from the viewpoint of its particular speciality, whereas a full appreciation of the subject requires the use of a variety of disciplines, *e.g.* cell biology, biochemistry, chemistry, enzymology, botany, and probably genetics.

An understanding of cell biology is required since our knowledge of the nature and functions of the amyloplast is limited, *e.g.* the amyloplasts of potato tubers turn into chloroplasts when exposed to light for a few days.[94] Is the amyloplast a non-photosynthetic chloroplast? If so, what eliminates or stimulates photosynthetic character in a plastid?

Cell biology and biochemistry overlap in consideration of the possible functions of the Golgi bodies and the mitochondria in the synthesis or degradation of starch. The Golgi bodies are suspected to be involved in the synthesis of cell wall material such as cellulose. Could the function of the α- and β-amylases be to degrade starch to provide substrates for this synthesis? Do ADP and UDP diffuse from the plastid to a mitochondrion for their conversion into energy-rich ATP and UTP, or can the plastid perform this function itself?

The chemistry of the separated amylose and amylopectin components has been extensively studied but problems still remain. For example, little is known of the conditions which surround the precipitation of the granular layers from the polysaccharide material in solution. This could perhaps be described as a ternary polymer–solvent precipitation.[95]

The problems in enzymology are concerned with the possible detection of new enzymes and with the elucidation of the action patterns of some of the known enzymes. Of particular interest is the nature of the association of transglucosylase enzyme with the surface of the starch granule and the effect of this upon synthesising ability. The feasibility of surface-labelling the starch granule is worthy of investigation in the former case.[96]

The requirement for botany is self-explanatory; it could also act as a guide to a genetic study. The relationship of genes to the presence of specific enzymes within the plastid could be a very significant field of investigation.[22]

In conclusion, the statement made by Professor Badenhuizen nearly ten years ago still applies:[24] 'There are still wide gaps in our knowledge of the starch granule, but to fill them is an attractive task for biologist and chemist alike.' Perhaps some gaps have been closed in the last few years but others, previously unsuspected, have become obvious.

The author thanks Dr. C. T. Greenwood for originally arousing his interest in this subject. He also thanks Professor A. G. Ogston for encouragement and Dr. J. M. Gebicki for discussions.

[94] J. T. O. Kirk and R. A. E. Tilney-Bassett, 'The Plastids', Freeman and Co., London and San Francisco, 1967.
[95] K. Doi, *Biochim. Biophys. Acta*, 1965, **94**, 557.
[96] P. Nordin, H. C. Moser, and J. K. Senne, *Biochem. J.*, 1965, **96**, 336.