ENZYMIC SYNTHESIS OF POLYSACCHARIDES

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Introduction

Since the natural processes which lead to the synthesis of polysaccharides are frequently reversible under physiological conditions, most of the enzymes concerned can, under suitable circumstances, degrade polysaccharides to simpler substances. In any comprehensive review of these enzymes, it would be necessary to consider both their synthetic and their degradative functions, as well as their own physical and chemical properties. Such a review could not be condensed adequately into an article of this type, and so we shall lay emphasis on the mechanisms by which polysaccharides are synthesised, rather than on the enzymes responsible; further, we shall consider only the conversion of saccharides into larger molecules, without showing how the simpler sugars themselves arise, since this aspect was reviewed recently by Avison and Hawkins [1] in this series. Our aim will be to outline the present state of knowledge on the synthesis of each polysaccharide in turn, and then to show how a master pattern of synthesis is emerging in the field as a whole. One fundamental equation will be encountered frequently, *viz.*,

$$G_t\text{—O—X} + \text{H—O—}G_r \rightleftharpoons G_t\text{—O—}G_r + \text{X—O—H} \qquad (1)$$

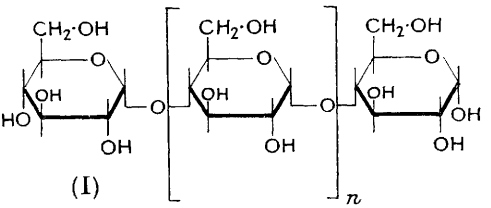
where G_tO and X are, respectively, the sugar residue and the aglycone portion (*i.e.*, the substituent at the reducing position) of a glycoside (G_t—O—X) which serves as the substrate for an enzyme, and G_r—O—H is a carbohydrate receptor molecule, the products being a higher saccharide (G_t—O—G_r) and a hydroxy-compound (X—O—H). The reader will see how each step in the synthesis of a higher saccharide always involves the transfer of the group G_t from OX to OG_r.

Synthesis of α-Glucosans of the Starch Class

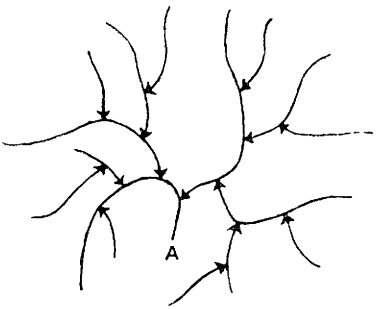
Most natural starches contain two macromolecular components, amylose and amylopectin, with the former constituting some 20—30% of the whole; a few starches, such as those derived from waxy maize and waxy sorghum, are exceptional inasmuch as they are practically devoid of amylose, while in others, such as wrinkled pea starch, amylose is the principal constituent. Amylose (I) is a polyglucose in which the sugar residues are joined by 1 : 4 α-linkages to form chains several hundred units in length; there is little or no branching of the chains. In amylopectin (II) short chains of the amylose type, averaging about 20 glucose units in length, are joined at branch points, principally by 1 : 6-α-linkages, each molecule containing more than a thousand glucose units altogether.

[1] *Quart. Reviews*, 1951, **5**, 171.

Amylose from Glucose-1 Phosphate.—It is appropriate that the phosphorylase-catalysed synthesis of amylose from dipotassium glucose-1 phosphate should be our first consideration, because this was the first enzymic synthesis of a polysaccharide *in vitro* to be established conclusively. In 1937, Cori, Colowick, and Cori [2] showed that a salt of α-glucopyranose-1 (dihydrogen phosphate) was formed when a solution of glycogen, inorganic



(I)



Reducing end-group is at A

(II) Meyer's structure for amylopectin

phosphate, and adenylic acid was incubated with a dialysed muscle extract. Subsequent investigations by the same authors [2] and by Cori, Schmidt, and Cori [3] using muscle phosphorylase, by Schäffner and Specht [4] and by Kiessling [5] with yeast phosphorylase, by Ostern, Herbert, and Holmes [6] with liver phosphorylase, and by Hanes [7] with phosphorylases from peas and potatoes, soon established that the reaction was reversible ; it can be represented in the following overall equation :

$$n\mathrm{C_6H_{11}O_5{\cdot}O{\cdot}PO_3K_2} \rightleftharpoons \underset{1:4\text{-}\alpha\text{-glucosan}}{(\mathrm{C_6H_{10}O_5})_n} + n\mathrm{K_2HPO_4} \quad . \qquad \text{(ii)}$$

Phosphorylase is now known to be very widespread in Nature ; in addition to the above sources, it has been found, for example, in waxy maize,[8] barley,[9]

[2] *J. Biol. Chem.*, 1937, **121,** 465 ; 1938, **123,** 375, 381.
[3] *Science*, 1939, **89,** 464.
[4] *Naturwiss.*, 1938, **26,** 494 ; 1939, **27,** 195.
[5] *Ibid.*, 1939, **27,** 129 ; *Biochem. Z.*, 1939, **302,** 50.
[6] *Nature*, 1939, **144,** 34 ; *Biochem. J.*, 1939, **33,** 1858.
[7] *Nature*, 1940, **145,** 348 ; *Proc. Roy. Soc.*, 1940, *B*, **128,** 421 ; **129,** 174.
[8] Bliss and Naylor, *Cereal Chem.*, 1946, **23,** 177.
[9] Porter, *Biochem. J.*, 1949, **45,** xxxvii.

Lima beans,[10] jack beans,[11] broad beans,[12] sugar beet,[13] and in the micro-organisms, *Neisseria perflava* [14] and *Polytomella coeca*.[15]

At equilibrium, the ratio of total inorganic phosphate to total glucose-1 phosphate is dependent on the pH value of the system, but the ratio of the bivalent ions, $[HPO_4]^{--}/[C_6H_{11}O_5{\cdot}O{\cdot}PO_3]^{--}$, is independent of pH and is always constant at 2·2.7* Thus, the conversion of an unbranched 1 : 4-α-glucosan into glucose-1 phosphate can be carried to virtual completion if the polysaccharide is treated with phosphorylase in the presence of a sufficiently large excess of inorganic phosphate to ensure that the equilibrium ratio of the bivalent ions is not attained before all the polysaccharide is degraded.[16–18] On the other hand, only about 35—40% of amylopectin can be phosphorylated in this way, because phosphorylase, which acts by removing successive glucose units at non-reducing chain ends, cannot break or by-pass the 1 : 6-linkages which constitute the branch points ; the action of the enzyme ceases when the outer chains of the main branches of the polysaccharide have been shortened to 3—6 glucose units.[17–21] It is interesting that arsenate can replace phosphate in these degradations and that the glucose-1 arsenate so formed is immediately hydrolysed to glucose. Because of this instability of the arsenate ester, no arsenate–glucose-1 arsenate equilibrium, analogous to that found in the phosphate case, can be established, and so the arsenolytic reaction results in the complete degradation of unbranched polysaccharides containing only 1 : 4-α-linkages, even when only traces of arsenate are employed.[20, 22, 23]

Nature of the Synthetic Polysaccharide.—When Hanes [7] synthesised an amylosaccharide *in vitro* from glucose-1 phosphate by the agency of potato phosphorylase, he recognised that the product differed from natural potato starch inasmuch as it was less soluble in water, was stained more deeply blue by iodine, and gave a higher yield of maltose (95—100% compared with *ca.* 60%) when treated with β-amylase ; in fact the synthetic polysaccharide showed a close resemblance to the " amyloamylose " (amylose) component of potato starch, prepared by the early fractionation method of Samec and Mayer.[24] Likewise, Cori, Schmidt, and Cori [3] had observed that,

[10] Green and Stumpf, *J. Biol. Chem.*, 1942, **142**, 355.
[11] Sumner, Somers, and Sisler, *ibid.*, 1944, **152**, 479.
[12] Hobson, Whelan, and Peat, *J.*, 1950, 3566.
[13] Kursanov and Pavlinova, *Biokhim.*, 1948, **13**, 378.
[14] Hehre, Hamilton, and Carlson, *J. Biol. Chem.*, 1949, **177**, 267.
[15] Lwoff, Ionesco, and Gutmann, *Biochim. Biophys. Acta*, 1950, **4**, 270.
[16] Swanson, *J. Biol. Chem.*, 1948, **172**, 805, 825.
[17] Bourne, Sitch, and Peat, *J.*, 1949, 1448.
[18] Hestrin, *J. Biol. Chem.*, 1949, **179**, 943.
[19] Meyer and Bernfeld, *Helv. Chim. Acta*, 1942, **25**, 399, 404.
[20] Katz, Hassid, and Doudoroff, *Nature*, 1948, **161**, 96.
[21] Cori and Larner, *J. Biol. Chem.*, 1951, **188**, 17.
[22] Katz and Hassid, *Arch. Biochem.*, 1951, **30**, 272.
[23] Meyer, Weil, and Fischer, *Helv. Chim. Acta*, 1952, **35**, 247.
[24] *Kolloidchem. Beih.*, 1921, **18**, 272.

* For a recent detailed study of this equilibrium see Trevelyan, Mann, and Harrison, *Arch. Biochem.*, 1952, **39**, 419, 440.

whereas the natural amylosaccharide of the animal body is glycogen (a polyglucose similar in structure to amylopectin, but more highly branched), which is stained red-brown by iodine, muscle phosphorylase synthesises *in vitro* a polysaccharide giving an intense blue stain. Subsequently, polysaccharides synthesised *in vitro* by phosphorylases derived from a variety of sources were submitted to methylation and end-group assay,[25-28] to determinations of molecular weight,[25, 29] to colorimetric assays when stained with iodine,[17, 30, 31] to potentiometric titrations with iodine,[32] and to β-amylolysis.[7, 27, 33, 34] These methods, chosen because they distinguish clearly between amylose, on the one hand, and amylopectin and glycogen, on the other, proved beyond doubt that the synthetic product was always an unbranched polyglucose of the amylose type. Thus it can be seen that phosphorylase is specific for both the synthesis and phosphorolysis of 1 : 4-α-glucosidic linkages, and cannot be solely responsible for the formation of amylopectin and glycogen; as will be shown later, a supplementary enzyme is necessary in the synthesis of each of these branched polysaccharides.

Conditions and Mechanism of the Synthetic Reaction.—So far as is known at present, α-D-glucose-1 phosphate is the only substrate on which phosphorylase can display its synthetic function; the enzyme has no action on the β-anomer,[35] or on the 1-phosphates of α-L-glucose,[36] α-D-galactose,[3, 37] α-D-mannose,[3, 37] α-D-xylose,[37] α-maltose,[37] or α-D-glucuronic acid.[37a] Hanes [7] observed that there was an induction period when potato phosphorylase was incubated with glucose-1 phosphate which had been prepared from starch by phosphorolysis; the addition of a little starch abolished this lag phase. A similar observation had been made by Cori and Cori [38] using muscle phosphorylase; the effect was more marked with the more highly purified enzyme samples. By using chemically synthesised glucose-1 phosphate, Green and Stumpf [10] were able to extend indefinitely the lag phase shown by specially purified potato phosphorylase, but synthesis could again be initiated by the introduction of starch or dextrins derived from starch. Thus it became apparent that a "primer" is necessary for the synthesis of amylose, but that, unless special precautions are taken in the

[25] Hassid and McCready, *J. Amer. Chem. Soc.*, 1941, **63**, 2171.
[26] Haworth, Heath, and Peat, *J.*, 1942, 55.
[27] Hassid, Cori, and McCready, *J. Biol. Chem.*, 1943, **148**, 89.
[28] Barker, Bourne, and Wilkinson, *J.*, 1950, 3027.
[29] Haworth, Heath, and Peat, unpublished result mentioned in *J.*, 1945, 877.
[30] Hassid and McCready, *J. Amer. Chem. Soc.*, 1943, **65**, 1154, 1157.
[31] Bear and Cori, *J. Biol. Chem.*, 1941, **140**, 111.
[32] Bates, French, and Rundle, *J. Amer. Chem. Soc.*, 1943, **65**, 142.
[33] Bourne and Peat, *J.*, 1945, 877.
[34] Barker, Bourne, Peat, and Wilkinson, *J.*, 1950, 3022.
[35] Wolfrom, Smith, Pletcher, and Brown, *J. Amer. Chem. Soc.*, 1942, **64**, 23; Wolfrom, Smith, and Brown, *ibid.*, 1943, **65**, 255.
[36] Potter, Sowden, Hassid, and Doudoroff, *ibid.*, 1948, **70**, 1751.
[37] Meagher and Hassid, *ibid.*, 1946, **68**, 2135.
[37a] Barker, Bourne, Fleetwood, and Stacey, unpublished results.
[38] *J. Biol. Chem.*, 1939, **131**, 397.

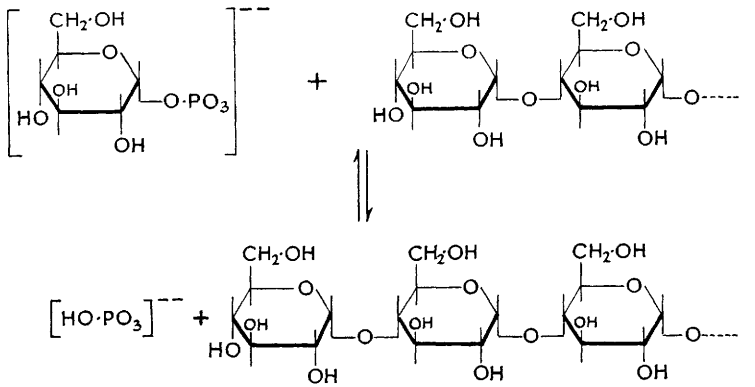
purification of the enzyme and of the phosphate ester, there is usually sufficient primer present as an impurity to initiate the synthesis. It is now known that the primer must be a glucose " polymer ", with 1 : 4-α-links ; for example, glucose,[7, 10] fructose,[7] sucrose,[7, 10] and dextran [17, 18, 39] do not function in this way.

A more precise definition of the essential structural features of the primer cannot be given in a general statement, because different phosphorylases have different requirements, as can be seen by considering the phosphorylases of the potato, jack bean, and muscle. In the case of potato phosphorylase, the molecular size of the primer is not critical, because, although polysaccharide synthesis is not promoted by maltose,[10, 17] the higher linear homologues of maltose containing three, four, five, or six glucose units are effective,[39, 40] as also are starch, amylose, and amylopectin.[7, 10, 17] Comparison of the relative efficiencies of 1 : 4-α-glucosans as primers for the potato enzyme has shown that there are at least two controlling factors. First, priming power is related to the number of non-reducing end groups available ; this explains (*a*) why amylopectin (5% of end groups) is more effective than amylose (< 0·5% of end groups),[17] (*b*) why in the early stages of the acidic hydrolysis of amylose and amylopectin there is a rapid increase in priming power,[17, 41, 42] and (*c*) why the cyclic Schardinger dextrins, which contain 6—8 glucose units linked by 1 : 4-α-bonds, are devoid of priming activity.[10, 43] Since oxidation of the terminal aldehydic grouping has little effect on the priming ability of a short unbranched dextrin of the amylose type, the presence of a reducing end group cannot be a factor contributing to the priming properties.[18] Secondly, the effects of acidic hydrolysis and of β-amylolysis on the ability of amylose to function as a primer for potato phosphorylase cannot be explained simply on the increased availability of non-reducing terminal glucose units, and it seems that there is a certain length of chain at which priming activity reaches an optimum ; this chain length is probably about 20 glucose units.[17] Muscle phosphorylase resembles potato phosphorylase inasmuch as it displays its synthetic activity only in the presence of a primer containing non-reducing terminal glucose units ; it is probably for this reason that glycogen (9% of end groups) is a much more efficient primer for the muscle enzyme than is amylose (< 0·5% of end groups).[27, 38, 44–46] On the other hand, muscle phosphorylase requires these end groups to be supplied as part of a macromolecule, as is shown by two facts : (*a*) that it is not primed by higher homologues of maltose containing fewer than eight glucose units,[46] and (*b*) that the priming power of glycogen for the enzyme is rapidly destroyed when the polysaccharide is treated mildly with acid, in spite of the fact that such a treatment increases

[39] Weibull and Tiselius, *Arkiv Kemi, Min., Geol.*, 1945, **19**, *A*, No. 19.
[40] Bailey, Whelan, and Peat, *J.*, 1950, 3692.
[41] Hidy and Day, *J. Biol. Chem.*, 1944, **152**, 477 ; 1945, **160**, 273.
[42] Swanson and Cori, *ibid.*, 1948, **172**, 815.
[43] Proehl and Day, *ibid.*, 1946, **163**, 667.
[44] Cori and Cori, *Ann. Rev. Biochem.*, 1941, **10**, 152.
[45] Cori, Cori, and Green, *J. Biol. Chem.*, 1943, **151**, 39.
[46] Cori, Swanson, and Cori, *Fed. Proc.*, 1945, **4**, 234.

the number of non-reducing terminal glucose units.[42, 46] Jack-bean phosphorylase differs in its primer requirements from both the muscle and potato enzymes, for it is primed more efficiently by amylose than by amylopectin.[47]

So far as is known at present, all phosphorylases catalysing the conversion of glucose-1 phosphate into amylose require the presence of a 1 : 4-α-glucosan primer containing non-reducing chain ends, but differ as regards the most suitable molecular size for the primer. It is not surprising that there should be minor differences of this sort, because it is well established that the enzymes themselves are not identical chemically, as can be seen from the following three examples. First, muscle phosphorylase can be obtained readily in crystalline form,[48] whereas potato phosphorylase has so far not been crystallised, in spite of attempts by many workers to do so ; recently,



(iii)

a much improved method for the purification of potato phosphorylase has been devised [49] so that the chances of crystallising the enzyme have improved. Secondly, the phosphorylases of muscle [48] and adipose tissue [50] require adenylic acid before they display their full activity, whereas those of the potato [10] and the jack bean [47] do not. Thirdly, glucose is a competitive inhibitor in the case of synthesis by muscle phosphorylase [45] but not by jack-bean phosphorylase.[47]

The mechanism now generally accepted for the synthesis of amylose from salts of glucose-1 phosphate is that advanced by the Cori's and their school,[44–46] largely on the basis of the part played by non-reducing end groups in the priming reactions mentioned above. Each step in the synthesis is pictured as shown above.

It will be seen that this equilibrium is a special case of the general equation (i) ; one molecule of glucose-1 phosphate (G_t—O—X) reacts with

[47] Sumner, Chou, and Bever, *Arch. Biochem.*, 1950, **26**, 1.
[48] Green, Cori, and Cori, *J. Biol. Chem.*, 1942, **142**, 447.
[49] Gilbert and Patrick, *Biochem. J.*, 1952, **51**, 186.
[50] Creasey and Gray, *ibid.*, 1951, **50**, 74.

a 1 : 4-α-glucosan receptor molecule (H—O—G_r), to form a glucosan containing one additional glucose residue (G_t—O—G_r), together with mineral phosphate (X—O—H). Thus the function of primers is to serve as receptors for glucose residues, which become attached step-wise at the non-reducing ends; they are not true catalysts, but enter stoicheiometrically into the reaction. This mechanism explains why the average chain length of the synthetic amylose is dependent on the ratio of terminal receptor sites to glucose-1 phosphate molecules converted; a high proportion of the ester phosphate yields a long-chain polymer and a small proportion gives a short-chain product.[11, 16, 51] It follows that phosphorylase catalyses the *simultaneous* lengthening of all pre-formed chains in the receptor molecules and does not lengthen one chain to its full extent before dealing with the remaining chains.[16, 51, 52]

Before turning to other enzyme systems capable of synthesising amylosaccharides, it is interesting to note the truly fantastic speed at which phosphorylase performs its highly specific task; Cori, Cori, and Green [45] have calculated that a mole of enzyme transforms 4×10^4 moles of glucose-1 phosphate per minute under optimum conditions!

Amylose from Maltose.—Monod and Torriani [53–55] have described the synthesis of an iodophilic polysaccharide from maltose by means of a cell-free extract of *Escherichia coli* (Monod strain ML). They have given the name "amylomaltase" to the enzyme responsible, and have shown that it catalyses the following reversible overall reaction:

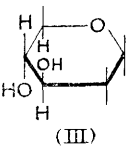
$$n \text{ Maltose} \rightleftharpoons (\text{Glucose})_n + n \text{ Glucose} \quad . \quad . \quad \text{(iv)}$$

It is an adaptive enzyme inasmuch as it is produced only when the organism is grown on maltose and not, for example, on glucose or lactose; [54] it shows a high measure of substrate specificity, being without action on methyl α- or β-D-glucoside, cellobiose, lactose, sucrose, melibiose, or glucose-1 phosphate.[53] In the forward reaction, equilibrium is normally established when 60% of the maltose has been converted, and at this stage the polymeric product gives a faint red stain with iodine, suggesting that the average chain length is about ten glucose units.[53–55] If, however, the synthesis is conducted in the presence of notatin (glucose oxidase), the conditions of equilibrium can never be established, and the conversion of maltose proceeds to completion.[53, 55] The polyglucose thus obtained is probably amylose since it gives a blue stain with iodine,[53] but a full structural analysis has not yet been made.

The reverse reaction proceeds when the synthetic polysaccharide is incubated with amylomaltase in the presence of glucose, as is shown by the diminished intensity of the blue iodine stain given by the digest, and by the appearance of a second reducing sugar (maltose ?); [53, 56] in the absence of

[51] Bailey and Whelan, *Biochem. J.*, 1952, **51,** xxxiii.
[52] Bourne and Whelan, *Nature*, 1950, **166,** 258.
[53] Monod and Torriani, *Compt. rend.*, 1948, **227,** 240; 1949, **228,** 718.
[54] Monod, *Biochem. Soc. Symposia*, 1950, No. 4, 51.
[55] Monod and Torriani, *Ann. Inst. Pasteur*, 1950, **78,** 65.
[56] Doudoroff, Hassid, Putman, Potter, and Lederberg, *J. Biol. Chem.*, 1949, **179,** 921.

glucose, the enzyme does not attack the polysaccharide, a fact which distinguishes it from the amylases.[53] Indications have been obtained that D-xylose and D-mannose, but not D-fructose, D-galactose, D-arabinose, or L-arabinose, can replace D-glucose in this reversal of the synthesis to yield analogues of maltose.[56] Verification of this would suggest that enzyme specificity towards this particular component in the reaction is determined by the presence or absence of the structure (III).


(III)

Additional evidence that amylomaltase occurs in *E. coli* which has been grown on maltose has been obtained from studies of the extra-cellular saccharides formed when washed resting cells of the organism are incubated with maltose, in the presence of iodoacetate and in the absence of notatin.[56, 57] Using a mutant of *E. coli* (strain W-327), Hassid and his co-workers [56] obtained glucose, unchanged maltose, and a series of dextrins containing 4—6 glucose units per molecule; the presence of 1 : 4-α-linkages in the dextrins was strongly indicated by their susceptibility to β-amylolysis. In a similar experiment with Monod's strain (ML) of *E. coli*, Barker and Bourne [57] fractionated the saccharides on a charcoal column and proved by both chemical and biochemical methods that they consisted of glucose, unchanged maltose, and higher homologues of maltose (3—5 glucose units in length). Of the glucose residues present initially in the maltose, approximately 29% appeared as free glucose, 24% as unchanged maltose, and 35% as higher saccharides.

Although there are still several problems connected with amylomaltase-catalysed reactions which merit further study, it seems probable [53–57] that each step in the synthesis of amylose entails the transfer of a $C_6H_{11}O_5$ unit [G_t; see equation (i)] from maltose (G_t—O—X) to an amylosaccharide molecule (H—O—G_r) with the elimination of a molecule of glucose (X—O—H), as follows:

$$\left.\begin{array}{rcl} \text{Maltose} + \text{Maltose} & \rightleftharpoons & \text{Maltotriose} + \text{Glucose} \\ & & \text{(first step)} \\ \text{Maltose} + (\text{Glucose})_n & \rightleftharpoons & (\text{Glucose})_{n+1} + \text{Glucose} \\ & & \text{(later step)} \end{array}\right\} \quad . \quad \text{(v)}$$

It has not yet been established whether the glucose unit (G_t), which is transferred by amylomaltase to the receptor molecule, must be furnished as maltose; it is possible, for example, that equation (v) is really a special case and that each step in the reaction could be written in the more general form (where $x > 1$):

$$(\text{Glucose})_x + (\text{Glucose})_n \rightleftharpoons (\text{Glucose})_{n+1} + (\text{Glucose})_{x-1} \quad . \quad \text{(vi)}$$

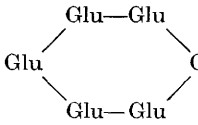
Furthermore, it is possible that more than one glucose residue can be transferred at any one time. Indeed, the fact that, in the presence of notatin, a very considerable increase (> 4-fold) in the chain length of the product results, although the conversion of maltose is increased only from 60% to

[57] Barker and Bourne, *J.*, 1952, 209.

100%, may be attributable to this. Alternatively, it may be due to a greater affinity of the enzyme for longer chains.

Linear Amylosaccharides from Cyclic Amylosaccharides.—In 1905, Schardinger [58] showed that, during the cultivation of *B. macerans* on starch, non-reducing crystalline saccharides (Schardinger dextrins) are produced. It is now known that such dextrins are *cyclo*amyloses, *i.e.*, that each dextrin molecule contains a loop of glucose units, mutually linked by 1 : 4-α-bonds; the α-, β-, and γ-Schardinger dextrins contain, respectively, six, seven, and eight glucose units per molecule.[59] The extra-cellular enzyme responsible for the formation of these dextrins was first isolated by Tilden and Hudson,[60] and has now been obtained in an electrophoretically pure form.[61, 62] The dextrins probably arise mainly from the amylose component of the starch substrate and from the outer chains of amylopectin.[63, 64]

The early classification of the enzyme as an amylase was unfortunate because, as Cori pointed out,[65] its action is not hydrolytic, since it involves the exchange of a glucosidic linkage in a polysaccharide chain for a similar one in a cyclic dextrin. The small ΔF which would accompany such an exchange led to the belief that the reaction should be readily reversible, as indeed has been demonstrated in the following case (Glu = a glucose unit) : [64, 66]



$$\text{Glu}+\text{Glu—Glu} \rightleftharpoons \text{Glu—[Glu]}_6\text{—Glu (+Homologues)} \quad \text{(VII)}$$

In analogous reactions, the maltose component can be replaced by glucose, methyl α-D-glucoside, sucrose, cellobiose, or maltobionic acid.[66] More recently, Norberg and French [67] have shown that the activity of the *Bacillus macerans* enzyme is not limited to reactions involving Schardinger dextrins, but that such reactions really represent one aspect of a more general reaction. They found that the enzyme catalysed a redistribution of the glucose residues in linear amylosaccharides ; from maltose, for example, they obtained a series of oligosaccharides, thus :

$$\left.\begin{aligned} 2\text{Glu}_2 &\rightleftharpoons \text{Glu}_1 + \text{Glu}_3 \\ \text{Glu}_2 + \text{Glu}_3 &\rightleftharpoons \text{Glu}_1 + \text{Glu}_4 \ \ldots \ \text{etc.} \end{aligned}\right\} \quad \text{(VIII)}$$

Overall reaction :

$$n\text{Glu}_2 \rightleftharpoons x\text{Glu}_1 + y\text{Glu}_2 + z\text{Glu}_3 + \text{higher amylosaccharides}$$

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[58] *Zentr. Bakt.*, II, 1905, **14**, 772 ; 1909, **22**, 98.
[59] Freudenberg and Cramer, *Ber.*, 1950, **83**, 296.
[60] *J. Amer. Chem. Soc.*, 1939, **61**, 2900.
[61] Schwimmer and Garibaldi, *Cereal Chem.*, 1952, **29**, 108.
[62] Schwimmer, *Fed. Proc.*, 1952, **11**, 283.
[63] Wilson, Schoch, and Hudson, *J. Amer. Chem. Soc.*, 1943, **65**, 1380.
[64] Myrbäck and Willstaedt, *Acta Chem. Scand.*, 1949, **3**, 91.
[65] *Fed. Proc.*, 1945, **4**, 226.
[66] French, Pazur, Levine, and Norberg, *J. Amer. Chem. Soc.*, 1948, **70**, 3145.
[67] *Ibid.*, 1950, **72**, 1202, 1746.

A similar redistribution occurred with amyloheptaose, the synthesis of cyclic dextrins being more apparent, of course, in this case.[67] The individual reactions in the series proceed at different rates, as can be seen from two facts: (*a*) amyloheptaose is converted much more readily than is maltose, and (*b*) the α-, β-, and γ-Schardinger dextrins are formed at different speeds.[67] The formation of cyclic dextrins is facilitated by the natural tendency for a chain of 1 : 4-α-glucose units to assume a helical configuration, and also because a 1 : 4-α-linkage in such a cyclic structure is slightly more stable than is a similar bond in a linear dextrin.[68]

Although amylomaltase and the *B. macerans* enzyme differ inasmuch as it has been reported [53, 63] that the latter, but not the former, degrades starch in the absence of glucose, they show remarkable similarities in their actions on the lower amylosaccharides [108] [compare equations (v) and (viii)]. A closer comparison between the enzymes would make an interesting study; two problems which might thus be solved are (*a*) whether cyclic dextrins occur in the products of the amylomaltase-catalysed conversion of linear dextrins, and (*b*) whether the *B. macerans* enzyme can synthesise a polysaccharide of the amylose type from maltose in the presence of notatin.

Amylopectin from Amylose.—Several mechanisms for the synthesis of unbranched 1 : 4-α-glucosans have now been discussed, and we must consider next how amylopectin, the branched component of starch, might arise. In 1944, Haworth, Peat, and Bourne [69] announced the isolation from potato juice of an enzyme fraction which synthesised a polysaccharide, giving a reddish-purple iodine stain, from glucose-1 phosphate, in the presence, but not in the absence, of potato phosphorylase. The active principle of this fraction, termed Q-enzyme, was obtained later in a purer state by an improved method of isolation.[70] Gilbert and Patrick [71] subsequently crystallised Q-enzyme, after a carefully investigated purification procedure, involving precipitation with ethanol at low temperature from solutions of low ionic strength. The nature of the polysaccharide synthesised by the joint action of these two enzymes of the potato is dependent on the relative activities of the enzymes.[34] When a high proportion of Q-enzyme is employed, the product is indistinguishable from natural potato amylopectin in its iodine staining properties (blue value, *ca.* 0·12), in the rate and extent (*ca.* 55%) of its conversion into maltose by β-amylase, in its ability to prime the synthesis of amylose from glucose-1 phosphate (see p. 60), and in its average chain length (*ca.* 20 glucose units) as determined by methylation and end-group assay; [28, 33, 34, 69] it does, however, have a somewhat smaller molecular weight than the native polysaccharide,[33] but this is not surprising in view of the vastly different conditions attending their formation. Hydrolysis of the methylated polysaccharide, as of tri-*O*-methylamylopectin, affords 2 : 3 : 4 : 6-tetra-*O*-methylglucose (from the non-reducing terminal units),

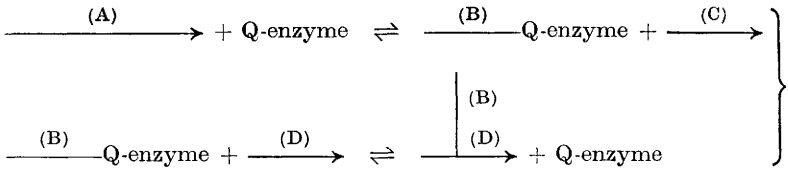
[68] Myrbäck, *Arkiv Kemi, Min., Geol.*, 1949, **1**, 161.
[69] *Nature*, 1944, **154**, 236.
[70] Barker, Bourne, and Peat, *J.*, 1949, 1705.
[71] *Nature*, 1950, **165**, 573, 878; *Biochem. J.*, 1952, **51**, 181.

2 : 3 : 6-tri-*O*-methylglucose (from units within the chains), and 2 : 3-di-*O*-methylglucose (from the branch points); thus the principal glucosidic linkages involve positions 1 and 4, while the branch linkages are of the 1 : 6-type.[28] When the synthesis from glucose-1 phosphate is catalysed by mixtures of phosphorylase and Q-enzyme containing higher proportions of the former enzyme, the properties of the resulting polysaccharides are intermediate between those of amylose and amylopectin.[28, 34]

The mechanism of potato Q-enzyme action has been determined from studies of its effect on amylose and starch. The product obtained from either of these substrates cannot be differentiated, except as regards molecular size,[72] from natural amylopectin by rigorous chemical and enzymic tests, similar to those described above.[70–76] In contrast to the α- or β-amylolysis of amylosaccharides, this amylose → amylopectin conversion entails the liberation of little or no reducing sugar (< 2%, expressed as maltose); [70–74] it is not a phosphorolysis since it proceeds equally well in the absence and presence of large proportions of inorganic phosphate,[77] provided that the Q-enzyme is already fully activated by the addition of salts, such as sodium acetate and ammonium chloride, to the digests.[77, 78] Thus it seems that Q-enzyme is a transglucosidase, operating by a non-phosphorolytic mechanism, which converts about one in every twenty 1 : 4-α-linkages of amylose into the 1 : 6-α-linkages which constitute the branch points of amylopectin [cf. equation (i)].[75, 77, 78a] It follows that the synthesis of amylopectin from glucose-1 phosphate by the joint action of phosphorylase and Q-enzyme is a two-stage process consisting of (1) the phosphorylase-catalysed synthesis of amylose-type molecules from the Cori ester, and (2) the conversion of these unbranched chains into amylopectin by Q-enzyme.[33, 75, 77, 79] Although the Q-enzyme samples used in the above studies were all obtained from the potato, it is probable that the enzyme is quite widespread in Nature; indeed similar Q-enzyme samples have been obtained already from the wrinkled pea,[12] the broad bean,[12] green gram,[80] *Neisseria perflava*,[14] and *Polytomella cœca*.[81, 82]

It is probable that each branch point in the amylopectin molecule is established according to the following mechanism, in which the arrows signify chains of 1 : 4-α-glucopyranose units, the reducing groups being indicated by the arrow-heads, and the branch points being of the 1 : 6-type:[83]

[72] Nussenbaum and Hassid, *J. Biol. Chem.*, 1951, **190**, 673.
[73] Bourne, Macey, and Peat, *J.*, 1945, 882.
[74] Peat, Bourne, and Barker, *Nature*, 1948, **161**, 127.
[75] *Idem*, *J.*, 1949, 1712.
[76] Cori and Illingworth, *J. Biol. Chem.*, 1951, **190**, 679.
[77] Barker, Bourne, Wilkinson, and Peat, *J.*, 1950, 93.
[78] Gilbert and Swallow, *J.*, 1949, 2849.
[78a] Hestrin, *Brewers' Digest*, 1948, **23**, 1.
[79] Hobson, Whelan, and Peat, *J.*, 1951, 596.
[80] Ram and Giri, *Arch. Biochem.*, 1952, **38**, 231.
[81] Bebbington, Bourne, Stacey, and Wilkinson, *J.*, 1952, 240.
[82] Bebbington, Bourne, and Wilkinson, *J.*, 1952, 246.
[83] **Barker, Bebbington, Bourne, and Stacey,** unpublished results.



(ix)

Studies with potato Q-enzyme have shown that the amylose-type substrate (A) must contain at least 42 glucose units before it is attacked by the enzyme.[84, 85] The initial attack probably involves fission of a 1 : 4-link in the substrate, with the formation of an amylosaccharide (B)–enzyme complex and a dextrin fragment (C). The complex could then react with a second amylosaccharide molecule (D) to give the branched product (BD), together with the free enzyme. The receptor molecule (D) might be, for example, an intact amylose molecule, the residual dextrin (C), or a branched product formed in an earlier stage of the reaction. Evidence that the molecular size of (D) is not important, at least in the case of the Q-enzyme of *Polytomella cœca*, was obtained recently,[86] when it was shown that the initial rate of conversion of amylose by dilute solutions of the enzyme, as measured by the fall in the blue value of the substrate, was markedly increased by the introduction of different amylopectins, glycogen, amylodextrins, or commercial maltose, but not by the cyclic Schardinger dextrins, or by carbohydrates devoid of the 1 : 4-α-glucosidic linkage, such as glucose, galactose, fructose, cellobiose, lactose, sucrose, dextran, inulin, and xylan. The function of these primers is presumably to increase greatly, in the early stages of the amylose conversion, the number of chains available as receptors of type (D). In the absence of such primers, the reaction of the protozoal enzyme is autocatalytic, since the conversion of amylose into amylopectin itself increases the number of receptor chains.[86] Although alternative explanations of these phenomena could be advanced, the above mechanism falls into line with polysaccharide syntheses in general.

The question of the reversibility of Q-enzyme action was examined by Barker, Bourne, Wilkinson, and Peat,[77] who were unable to find any conclusive evidence that the enzyme can break the 1 : 6-α-linkages of amylopectin or β-dextrin. It is clear that, under the experimental conditions so far employed, the equilibrium favours strongly the synthesis, rather than the fission, of the branch points. In fact, in this respect, Q-enzyme seems to be complementary to the R-enzyme of beans and potatoes, which can break, but not synthesise, the 1 : 6-α-linkages of amylopectin and related molecules;[87, 88] other 1 : 6-amyloglucosidases occur in muscle and in yeast.[19, 89–91]

[84] Bailey, Peat, and Whelan, *Biochem. J.*, 1952, **51,** xxxiv.
[85] Nussenbaum and Hassid, *J. Biol. Chem.*, 1952, **196,** 785.
[86] Barker, Bebbington, and Bourne, *Nature*, 1951, **168,** 834.
[87] Hobson, Whelan, and Peat, *Biochem. J.*, 1950, **47,** xxxix.
[88] *Idem, J.*, 1951, 1451.
[89] Cori and Larner, *Fed. Proc.*, 1950, **9,** 163; *J. Biol. Chem.*, 1951, **188,** 17.
[90] Maruo and Kobayashi, *J. Agric. Chem. Soc. Japan*, 1949, **23,** 115, 120.
[91] Petrova, *Biokhim.*, 1948, **13,** 244; 1951, **16,** 482.

In 1949, Beckmann and Roger [92, 93] showed that some of the characteristics of the Q-enzyme-catalysed conversion of amylose could be simulated by the addition of a fatty acid to the polysaccharide; they concluded that Q-enzyme was an artefact and that the "amylopectin" produced by its agency was really an amylose–fatty acid complex. This conclusion did not take account of methylation data presented four years earlier by Bourne and Peat,[33] and is at variance with much of the later work from the same school. Moreover, a method recommended by Beckmann and Roger, themselves,[92] for distinguishing between amylopectin and amylose–fatty acid complexes has revealed very close similarity between natural amylopectins, on the one hand, and our amylose conversion products (with potato, or *Polytomella cœca*, Q-enzyme), on the other.[81, 82] This method entails measurement of the spectra (2500—8000 Å) of iodine-stained solutions of the polysaccharides. Finally, Nussenbaum and Hassid [72] have shown the synthetic amylopectin to be devoid of fatty acid, and Cori and Illingworth [76] have confirmed, by means of a specific 1 : 6-amyloglucosidase, that 1 : 6-branch points are indeed present, and that the average chain length is 20 glucose units.

It has been claimed by Bernfeld and Meutémédian [94, 95] that amylopectin is produced from glucose-1 phosphate by the joint action of phosphorylase and an *iso*phosphorylase. This *iso*phosphorylase was believed to synthesise 1 : 6-α-glucosidic linkages from the Cori ester in a manner similar to that by which phosphorylase establishes 1 : 4-α-links. However, this claim can no longer be entertained, because (*a*) the mechanism of synthesis advanced by Bernfeld and Meutémédian is at variance with certain well-established principles of phosphorylase action,[77] (*b*) the experimental data can be interpreted quite adequately without having to postulate the existence of an *iso*phosphorylase,[96] and (*c*) neither Nussenbaum and Hassid [72] nor Meyer [97] could repeat the preparation of *iso*phosphorylase.

Glycogen from Glucose-1 Phosphate.—In view of the close relation between the structures of amylopectin and glycogen, it is not surprising that they should be synthesised by similar enzymic processes. The synthesis of glycogen from glucose-1 phosphate again requires two enzymes, phosphorylase to establish the 1 : 4-α-glucosidic bonds, and a supplementary enzyme ("branching factor") to form the branch points.[46, 50, 98–101] This supplementary enzyme, analogous to the Q-enzyme of the plant kingdom, has been isolated from several animal organs, such as the heart,[46, 98] the brain,[98] the liver,[46, 98, 99, 101] and adipose tissue.[50, 100] It was first reported by Cori and Cori [98] in 1943, the year before Q-enzyme was first described.[69]

Although the supplementary enzyme itself cannot utilise glucose-1 phos-

[92] Abstr. Amer. Chem. Soc. Meeting, New York, 1949, 36c.
[93] *J. Biol. Chem.*, 1951, **190**, 467.
[94] *Nature*, 1948, **162**, 297, 618.
[95] *Helv. Chim. Acta*, 1948, **31**, 1724, 1735.
[96] Bailey and Whelan, *J.*, 1950, 3573.
[97] Personal communication.
[98] Cori and Cori, *J. Biol. Chem.*, 1943, **151**, 57.
[99] Hestrin, *Brewers' Digest*, 1948, **23**, 1.
[100] Creasey and Gray, *Biochem. J.*, 1950, **46**, ix.
[101] Larner, *Fed. Proc.*, 1952, **11**, 245.

phate as a substrate, the liberation of mineral phosphate from the Cori ester by the joint action of muscle phosphorylase and the supplementary enzyme is autocatalytic, and is much faster than in the case of the phosphorylase alone.[46, 50, 98] This autocatalytic effect, which is shown also by mixtures of phosphorylase and Q-enzyme,[81, 102] and of phosphorylase and α-amylase,[95] was attributed by Cori and Cori [98] to the fact that the supplementary enzyme, by continually increasing the number of non-reducing chain ends, provides more " primers " for the phosphorylase (see p. 60).

An early observation that the supplementary enzyme is without action on amylose-type polysaccharides [98] (thus apparently differing from Q-enzyme in this most important respect) should now be re-examined, because Larner [101] has shown that the enzyme establishes, by a non-phosphorolytic transglycosidase mechanism, branch points in the outer chains of amylopectin, to give a product closely resembling glycogen in its iodine stain. Furthermore, an artificial polysaccharide, prepared from glycogen by lengthening the outer chains with ^{14}C-labelled glucose units (by means of phosphorylase and ^{14}C-labelled glucose-1 phosphate) was treated with the supplementary enzyme, and was then found to possess radioactivity at the new branch points.[101] It may be that the failure of the earlier enzyme to attack amylose was due to an insufficiency of receptor chains (see p. 67).

In an independent series of researches, Petrova [91, 103, 104] has studied a non-phosphorolytic enzyme fraction from rabbit muscle, termed by her " amylose isomerase ", which shows a very close resemblance to the supplementary enzyme of the Cori school inasmuch as it catalyses, jointly with phosphorylase, the synthesis of a glycogen-like product from glucose-1 phosphate. However, the isomerase seems to function also, in the reverse sense, as a 1 : 6-α-glucosidase, a property not apparently possessed by the supplementary enzyme. A more detailed experimental comparison between these two enzymes would be useful.

An Amylopectin-type Polysaccharide from Sucrose.—In 1946, Hehre and Hamilton [105, 106] reported that washed cells of *Neisseria perflava* synthesise a polyglucose (resembling amylopectin in its behaviour towards iodine, the amylases, and phosphorylase) from sucrose, but not from maltose, lactose, trehalose, melibiose, raffinose, melezitose, or methyl α-D-glucoside, or from a mixture of glucose and fructose. With glucose-1 phosphate a trace of an iodophilic polysaccharide was produced, but this synthesis, which was attributed to phosphorylase, was suppressed by the addition of excess of mineral phosphate, whereas that from sucrose was unimpaired. The same authors [106] found that 39 strains of *Neisseria perflava* all behaved similarly. From one of these strains (19-34), Hehre, Hamilton, and Carlson [14] isolated a cell-free enzyme, termed amylosucrase, which catalysed the conversion:

$$n \text{ Sucrose} \rightleftharpoons (\text{Glucose})_n + n \text{ Fructose} \quad . \quad . \quad (x)$$

[102] Barker, Bourne, Wilkinson, and Peat, *J.*, 1950, **84**.
[103] Petrova, *Biokhim.*, 1949, **14**, 155; 1952, **17**, 129.
[104] Petrova and Rozenfeld, *ibid.*, 1950, **15**, 309.
[105] *J. Biol. Chem.*, 1946, **166**, 777.
[106] *J. Bact.*, 1948, **55**, 197.

The amylosucrase was distinguished from the bacterial phosphorylase by its stability to heat and to gas treatment, and by the fact that the synthesis was not suppressed by phosphate.

The synthetic polysaccharide, which was virtually free from fructose, was shown to be a member of the amylopectin–glycogen class by α- and β-amylolysis, by phosphorolysis, by its iodine stain, by potentiometric titration with iodine, by its failure to give an insoluble butanol complex, and by negative serological tests for dextran. Through the kindness of Dr. Hehre, we were able to examine a polysaccharide produced by another strain (II-1) of *Neisseria perflava* and to confirm his conclusions regarding the structure : [107] methylation and end-group assay proved that chains of 1 : 4-α-glucopyranose units, averaging 11—12 units in length, were joined by branches of the 1 : 6-type. Since the synthetic polysaccharide possessed a branched structure, it seemed probable that the amylosucrase was contaminated with a second enzyme, which was responsible for the synthesis of the branch points, and this was verified when it was shown that the enzyme sample exhibited Q-enzyme activity inasmuch as it converted amylose into a glycogen-type polysaccharide without the appearance of reducing sugar.[14] Thus it was deduced that the function of amylosucrase itself is to convert sucrose into an unbranched polysaccharide of the amylose class, by a glucose-transferring mechanism involving the exchange of the biose linkage for a 1 : 4-α-glucosidic bond.[14] Although there is no direct evidence that amylosaccharide primers play an integral part in the reaction, as they do in the phosphorylase-catalysed synthesis of amylose, it is known that sucrose is not attacked by amylosucrase in the presence of α-amylase.[108]

Because the polysaccharide synthesis is strongly exothermic, a high conversion (*ca.* 98%) results, and the reverse reaction is difficult to demonstrate. Hehre and Hamilton [109] have, however, been able to show that a polysaccharide with the serological properties of dextran is formed in small yield (≡ 1% of sucrose) when a mixture of starch (or glycogen) and fructose is treated with amylosucrase and dextran sucrase [the latter enzyme converts sucrose into dextran (see below)].

It has been suggested [110, 111] that amylosucrase may play a part in the synthesis of an amylopectin-type polyglucose by cells of *Clostridium butyricum*, but acceptance of this hypothesis must await the results of experiments with cell-free extracts.

Synthesis of α-Glucosans of the Dextran Class

Dextran from Sucrose.—Dextrans are polyglucoses in which the majority of the bonds linking the sugar units are of the 1 : 6-α-type. They are syn-

[107] Barker, Bourne, and Stacey, *J.*, 1950, 2884.
[108] Hehre, *Adv. Enzymology*, 1951, **11**, 297.
[109] Hehre and Hamilton, *J. Biol. Chem.*, 1951, **192**, 161.
[110] Nasr and Baker, *Nature*, 1949, **164**, 745.
[111] Hobson and Nasr, *J.*, 1951, 1855.

thesised from sucrose, but not from glucose, by growing cultures of such micro-organisms as *Leuconostoc mesenteroides*, *Leuconostoc dextranicum*, and *Betabacterium vermiformé*. They show quite large variations in molecular structure; for example, the dextran produced by *Leuconostoc dextranicum* is an essentially unbranched polysaccharide, having an average chain length of 200—550 glucose units, whereas those from other organisms frequently possess a high degree of branching (average chain lengths 5—30 units).[112–117] The branch points are usually of the 1 : 4-type, but it has been shown recently that, in some cases, 1 : 3-linkages are involved.[118]

In 1941, Hehre [119] described the isolation from cultures of *Leuconostoc mesenteroides* of a heat-labile, cell-free extract, which synthesised from sucrose a polysaccharide, indistinguishable from a dextran by certain chemical and serological tests. He postulated that the synthesis, catalysed by dextran sucrase, proceeded according to the equation:

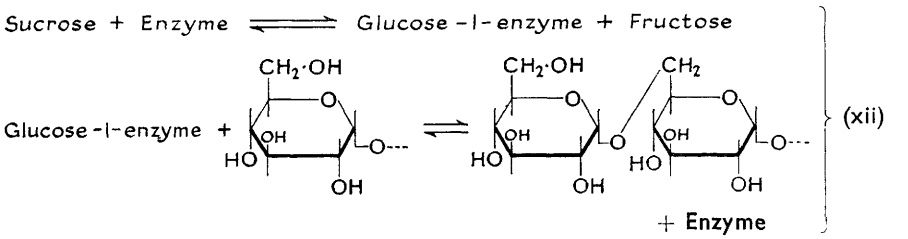
$$n \text{ Sucrose} \longrightarrow (\text{Glucose})_n + n \text{ Fructose} \quad . \quad . \quad \text{(xi)}$$

In later work by the same school, improved methods for the isolation of the enzyme were developed, and the optimum conditions for the synthesis were determined.[120–123] The enzyme was obtained free from "invertase", sucrose phosphorylase, and levan sucrase.[123] It was shown that the above equation was obeyed stoicheiometrically, and that only 0·1—1·2% of sucrose remained when equilibrium was reached; in the reverse reaction, no sucrose formation could be detected.[123] More recently, Forsyth and Webley [124, 125] have confirmed that dextran synthesis is overwhelmingly favoured at equilibrium (albeit with a final sucrose concentration of 8%), and have found also that traces of glucose are produced (the glucose was believed to arise from a hydrolytic process). Although all of the work described above was conducted with enzyme samples obtained from strains of *Leuconostoc mesenteroides*, other organisms,[120] such as lactobacilli, group H streptococci, and *Streptococcus salivarius*, are known to secrete dextran sucrase, but the enzymes from these sources have not yet been examined rigorously.

Since it has been shown that sugar phosphates are not formed as intermediates in the sucrose → dextran conversion,[108] and since the sucrose

[112] Peat, Schlüchterer, and Stacey, *J.*, 1939, 581.
[113] Daker and Stacey, *J.*, 1939, 585.
[114] Hassid and Barker, *J. Biol. Chem.*, 1940, **134**, 163.
[115] Levi, Hawkins, and Hibbert, *J. Amer. Chem. Soc.*, 1942, **64**, 1959.
[116] Stacey and Swift, *J.*, 1948, 1555.
[117] Jeanes and Wilham, *J. Amer. Chem. Soc.*, 1950, **72**, 2655.
[118] Barker, Bourne, Bruce, and Stacey, *Chem. Ind.*, 1952, 1156; Abdel-Akher, Hamilton, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1952, **74**, 4970; Lohmar, *ibid.*, p. 4974.
[119] Hehre, *Science*, 1941, **93**, 237.
[120] Hehre and Sugg, *J. Exp. Med.*, 1942, **75**, 339.
[121] Sugg and Hehre, *J. Immunol.*, 1942, **43**, 119.
[122] Hehre, *Proc. Soc. Exp. Biol. N.Y.*, 1943, **54**, 18.
[123] *Idem*, *J. Biol. Chem.*, 1946, **163**, 221.
[124] *Nature*, 1948, **162**, 150.
[125] *J. Gen. Microbiol.*, 1950, **4**, 87.

substrate cannot be replaced by a mixture of glucose and fructose,[119] it seems probable that each step in the synthesis of the 1 : 6-α-linked polyglucose chain must involve the exchange of the glucosidic link in sucrose for one in the polysaccharide, as follows :



There is no experimental proof that a receptor molecule (primer) is required to initiate the reaction, but this may be due to the fact that the enzyme has never been obtained free from associated dextran.[119, 120, 123] Alternatively, sucrose itself may serve as the primer, in which case terminal fructofuranose units should be present in the synthetic polysaccharide. The above scheme would lead, of course, to the formation of an unbranched dextran (a fact which has not yet been demonstrated experimentally), so that the problem of the mechanism by which the branches are established awaits solution ; it may well be that the branched polysaccharide is formed directly from the unbranched one by means of a second enzyme, as is the case with amylosaccharides.

In a recent paper,[126] Stodola *et al.* reported that a new *reducing* disaccharide, leucrose [5-*O*-(D-glucopyranosyl)-D-fructopyranose], is formed in about 3% yield during the synthesis of dextran by dextran sucrase isolated from *Leuconostoc mesenteroides*, and they postulated that this sugar " plays a role in the polymerisation process ". This conclusion, at variance with the above mechanism, which hitherto was widely accepted, would, if substantiated, throw grave doubts also on current theories regarding the synthesis of other polysaccharides from sucrose. Further studies of the problem are imperative ; they may show that the new disaccharide arises from a side-reaction in equation (xii), involving fructopyranose liberated in an earlier stage of the synthesis, namely :

$$\text{Glucose-1-enzyme} + \text{Fructopyranose} \rightleftharpoons \text{Leucrose} + \text{Enzyme} \qquad \text{(xiii)}$$

Dextran from Amylodextrins.—In an investigation of the phenomenon of " ropiness " in beer, a problem which had been studied at intervals for at least 50 years, Shimwell,[127] in 1947, demonstrated that cultures of *Acetobacter viscosum* and *Acetobacter capsulatum*, isolated from such beer, converted amylodextrins into highly viscous products ; these products were

[126] Stodola, Koepsell, and Sharpe, *J. Amer. Chem. Soc.*, 1952, **74**, 3202.
[127] *J. Inst. Brew.*, 1947, **53**, 280.

shown later by Hehre and Hamilton [128] to possess serological properties like those of certain dextrans. The organisms did not elaborate the slime when grown on glucose, fructose, sucrose, or maltose.[127] Hehre and Hamilton [128] obtained cell-free extracts of *Acetobacter capsulatum* which converted amylodextrins into a similar viscous material, and this product was studied in greater detail.[109] It was an amylase-resistant polyglucose, which did not stain with iodine, and was classified as a dextran on the basis of its serological properties, its stability towards acid, and its behaviour towards periodate; the ratio of 1 : 6-linkages to other glucosidic linkages (as revealed by the periodate oxidation) was *ca.* 5 : 1. The application of methylation techniques to a sample of this polysaccharide, kindly supplied by Dr. Hehre, has confirmed that the principal glucosidic bonds are of the 1 : 6-type, and has shown also that the molecules are branched and that the branches involve mainly positions 1 and 4.[129]

The enzyme responsible for the synthesis, dextrin-dextranase, cannot utilise, *inter alia*, maltose, sucrose, raffinose, or glucose-1 phosphate; nor are amylose, amylopectin and glycogen, or the higher dextrins which result therefrom by β-amylolysis, suitable as substrates. Indeed, the enzyme seems to require open-chain dextrins containing roughly 4—10 glucose units, such as are formed during the acidic hydrolysis or α-amylolysis of polysaccharides of the starch type.[109] From a study of the action of the enzyme on a purified sample of one of these dextrins, amyloheptaose, Hehre and Hamilton [109] concluded that the reaction involved the transfer of a glucose unit (in 1 : 4-α-linkage) from a non-reducing terminal position in an amylodextrin molecule to a corresponding position (in 1 : 6-α-linkage) in the growing dextran molecule, as follows (Glu = glucose unit):

$$\left.\begin{array}{l} \text{Glu 1—4 Glu 1—4 Glu 1}\cdots + \text{Glu 1—6 Glu 1}\cdots \\ \qquad\qquad\qquad\qquad \Updownarrow \\ \text{Glu 1—4 Glu 1}\cdots + \text{Glu 1—6 Glu 1—6 Glu 1}\cdots \end{array}\right\} \quad \text{(xiv)}$$

As partial confirmation of this mechanism, *cyclo*amyloheptaose was shown to be unattacked. They deemed further study desirable before it could be decided whether or not dextran-type molecules are necessary to initiate the reaction.

1 : 6-α-Linked Glucosaccharides from Maltose.—Following observations [130, 131] that the hydrolysis of starch by fungal amylases leads to the production of non-fermentable carbohydrates, Pan, Andreasen, and Kolachov [132, 133] found that a cell-free extract of *Aspergillus niger* (NRRL 337) converted maltose, but not glucose, into an unfermentable triose (panose), which was later obtained crystalline,[134] and which was proved by

[128] *Proc. Soc. Exp. Biol. N.Y.*, 1949, **71**, 336.
[129] Barker, Bourne, Bruce, and Stacey, unpublished results.
[130] Stark, *J. Biol. Chem.*, 1942, **142**, 569.
[131] Pigman, *J. Res. Nat. Bur. Stand.*, 1944, **33**, 105.
[132] *Science*, 1950, **112**, 115.
[133] *Arch. Biochem.*, 1951, **30**, 6.
[134] Pan, Nicholson, and Kolachov, *J. Amer. Chem. Soc.*, 1951, **73**, 2547.

French [135] and by Wolfrom *al.*[135a] to be *O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose.

A similar enzyme, obtained from *Aspergillus oryzæ*, has been examined in detail by Pazur and French who showed by chromatography,[136] and later by isolation of the products,[137] that maltose was transformed into *iso*maltose [6-*O*-α-D-glucosyl-D-glucose], *iso*maltotriose, panose, and a tetraose (containing two 1 : 6-α-linkages and one 1 : 4-α-linkage). Phosphorylation was apparently not involved since the enzyme could not utilise glucose, or glucose-1 phosphate, or a mixture of the two, and since phosphorylated intermediates did not arise in the synthesis from maltose.[137] Pazur and French postulated that the maltose conversion proceeded according to the following transglucosidase mechanism (Glu = glucose unit, E = enzyme) :

$$\left.\begin{array}{rcl} \text{Glu 1—4 Glu} + \text{E} & \rightleftharpoons & \text{Glu—E} + \text{Glu} \\ \text{Glu—E} + \text{Glu 1—4 Glu} & \rightleftharpoons & \text{Glu 1—6 Glu 1—4 Glu} + \text{E} \\ \text{Glu—E} + \text{Glu 1—6 Glu 1—4 Glu} & \rightleftharpoons & \text{Glu 1—6 Glu 1—6 Glu 1—4 Glu} + \text{E} \\ & & \text{etc.} \\ \text{Glu—E} + \text{Glu} & \rightleftharpoons & \text{Glu 1—6 Glu} + \text{E} \\ \text{Glu—E} + \text{Glu 1—6 Glu} & \rightleftharpoons & \text{Glu 1—6 Glu 1—6 Glu} + \text{E} \\ \text{Glu—E} + \text{Glu 1—6 Glu 1—6 Glu} & \rightleftharpoons & \text{Glu 1—6 Glu 1—6 Glu 1—6 Glu} + \text{E} \\ & & \text{etc.} \end{array}\right\} \text{(xv)}$$

Thus it can be seen why two series of oligosaccharides should be produced, in which the reducing units are attached through positions 4 and 6, respectively. The key reaction which leads to the latter series is the conversion of maltose and glucose into *iso*maltose and glucose, and as evidence of this, it was shown that a mixture of maltose and ^{14}C-glucose gave ^{14}C-*iso*maltose and ^{14}C-*iso*maltotriose, but inactive maltose and panose.[137] Evidently, the energy state of the glucose–enzyme complex is such that the free energy change in the direction of the synthesis of a 1 : 6-link is negative, while that for a 1 : 4-link is positive.[137] The above scheme is in agreement also with the order in which the different products appear.[137] The isolation from another strain of *Aspergillus oryzæ* of a transglucosidase, which also uses maltose as its substrate, and which is probably identical with that described above, has been reported by Wallenfels.[138]

More recently,[139] an enzyme from *Aspergillus niger* (strain 152) has been shown to convert maltose into a number of oligosaccharides, which have been proved by chromatography, and by chemical methods on purified fractions, to be identical with those described by Pazur and French.

Synthesis of Galactans

As far as we are aware, no enzymic synthesis of a polygalactose *in vitro* has yet been reported, but recent observations suggest a route by which

[135] *Science*, 1951, **113**, 352.
[135a] Wolfrom, Thompson, and Galkowski, *J. Amer. Chem. Soc.*, 1951, **73**, 4093.
[136] *Ibid.*, p. 3536.
[137] *J. Biol. Chem.*, 1952, **196**, 265.
[138] *Naturwiss.*, 1951, **38**, 306.
[139] Barker, Bourne, Carrington, and Stacey, unpublished results.

such a synthesis may shortly be achieved. Wallenfels and Bernt [138, 140] have claimed that a galactose-transferring enzyme, present in *Aspergillus oryzæ*, catalyses the following transformations of lactose [cf. equation (i)]:

$$\left.\begin{array}{rcl} \text{Gal 1—4 Glu} + \text{E} & \rightleftharpoons & \text{Gal—E} + \text{Glu} \\ \text{Gal—E} + \text{Lactose} & \rightleftharpoons & \text{Gal—Lactose} + \text{E} \\ \text{Gal—E} + \text{H}_2\text{O} & \longrightarrow & \text{Gal} + \text{E} \end{array}\right\} \quad . \quad . \quad \text{(xvi)}$$

In similar studies with lactases derived from *Saccharomyces fragilis* and *E. coli*, Aronson [140a] has confirmed these observations and has found that accompanying reactions are:

$$\left.\begin{array}{rcl} \text{Gal—E} + \text{Glu} & \rightleftharpoons & \text{Gal—Glu} + \text{E} \\ & & \text{(not lactose)} \\ \text{Gal—E} + \text{Gal} & \rightleftharpoons & \text{Gal—Gal} + \text{E} \end{array}\right\} \quad . \quad . \quad . \quad \text{(xvii)}$$

As would be expected from these equations, the first product is galactosyl-lactose, since lactose is the only receptor molecule present in significant amount in the early stages of the synthesis.[140a] In the presence of large amounts of glucose, lactose is transformed principally into an isomeric galactosyl-glucose. It seems that the transgalactosidation reaction involves a competition between water and receptor sugar molecules for the galactose–enzyme complex.[140a] The structures of the oligosaccharides have yet to be determined. It is probable that, if glucose were continuously removed from the system, higher saccharides, and possibly even polygalactans, would result. Indeed, Caputto and Trucco [141] have obtained from the mammary glands of rats, and also of cows, a series of oligogalactans, containing glucose; since lactose was detected in hydrolysates of these saccharides, the glucose residues must have been attached through $C_{(4)}$.

Synthesis of Fructosans of the Levan Class

It has been known for at least 50 years that certain micro-organisms are able to synthesise levans (*i.e.*, polyfructofuranoses in which the principal glycosidic linkages are of the 2 : 6-type) from sucrose. Before 1936, there were several reports that the synthesis had been effected with cell-free enzyme preparations, obtained from a culture filtrate of *Bacillus mesentericus vulgatus*,[142] from ruptured cells of the same organism,[143] from spore residues of *Aspergillus sydowi*,[144] from a sterile filtrate of *Bacillus subtilis*,[145] and from Oerskov's milk bacillus.[146] The fact that no clear picture of the mechanism of the synthesis had emerged by this time can be attributed to inadequate characterisation of the products in some of the cases cited, and to conflicting evidence concerning the nature of the polysaccharide precursor,

[140] *Angew. Chem.*, 1952, **64**, 28.
[140a] *Arch. Biochem. Biophys.*, 1952, **39**, 370.
[141] *Nature*, 1952, **169**, 1061.
[142] Beijerinck, *J. Soc. Chem. Ind.*, 1910, **29**, 710.
[143] Owen, *J. Ind. Eng. Chem.*, 1911, **3**, 481.
[144] Kopeloff, Kopeloff, and Welcome, *J. Biol. Chem.*, 1920, **43**, 171, 178.
[145] Harrison, Tarr, and Hibbert, *Canad. J. Res.*, 1930, **3**, 449.
[146] Dienes, *J. Infect. Dis.*, 1935, **57**, 12, 22.

which was believed by some workers to be sucrose itself, and by others to be " nascent " fructose (fructofuranose). Principally as a result of series of investigations by Hestrin and his colleagues,[147–152] it is now generally agreed that each step in the synthesis of levan, catalysed by levan sucrase, involves the following fructose transfer [cf. equation (i)]:

$$\text{Sucrose} + (\text{Fructose})_n = (\text{Fructose})_{n+1} + \text{Glucose} \quad . \quad . \quad \text{(xviii)}$$

Aschner, Avineri-Shapiro, and Hestrin [147, 148] first isolated the enzyme from *B. subtilis* by selective diffusion through an agar gel; they showed that it was an adaptive exocellular enzyme, *i.e.*, that it was present only when the bacillus was grown on a sucrose medium. An alternative method of isolation, more convenient for large-scale work, was based on autolysed cells of *Aerobacter levanicum* and yielded an active freeze-dried powder; in this case, the enzyme was constitutive and endocellular.[148] This enzyme produced levan from sucrose and raffinose, but not from invert sugar, maltose, lactose, trehalose, inulin, methyl $\alpha\beta$-D-fructofuranoside, glucose-1 phosphate, fructose-6 phosphate, or fructose-1 : 6 diphosphate.[149, 150] In the case of raffinose, the synthesis conformed with the equation:

$$\left.\begin{array}{r} n(\text{Gal 1—6 Glu 1—2 Fru}f) \\ \Updownarrow \\ (\text{Fru}f)_n + n(\text{Gal 1—6 Glu}) \\ (= \text{Melibiose}) \end{array}\right\} \quad . \quad . \quad . \quad . \quad \text{(xix)}$$

Since the levan sucrase was still active when free from phosphate, and the above phosphate esters were not substrates, it was deemed highly improbable that it could function by a phosphorolytic mechanism.[150] There was strong inhibition of levan synthesis from sucrose by D-glucose (competitive), D-galactose, D-xylose, L-arabinose, maltose, and lactose, but not by D-mannose, D-fructose, or D-glucosamine; it was concluded that the configuration at $C_{(2)}$ of a reducing sugar was the major factor in determining its inhibitory powers.[150] Some free fructose was always liberated in the synthesis from sucrose and raffinose, possibly owing to the presence of a hydrolase contaminant, or possibly because water may function as the receptor of the fructose unit in (xviii).[150, 152]

More recently, levan sucrase preparations possessing properties very similar to those described above have been obtained from *Streptococcus salivarius* and the spore-forming bacillus N9.[153, 154] The lævorotatory polyfructoses which were synthesised from sucrose by these preparations were classified as levans on the basis of serological tests.[153, 154] An interesting discovery, made when similar tests were applied to polysaccharides produced by a variety of streptococci, was that certain strains of group H

[147] Aschner, Avineri-Shapiro, and Hestrin, *Nature*, 1942, **149**, 527.
[148] *Idem*, *Biochem. J.*, 1943, **37**, 450.
[149] Hestrin and Avineri-Shapiro, *Nature*, 1943, **152**, 49.
[150] *Idem*, *Biochem. J.*, 1944, **38**, 2. [151] Hestrin, *Nature*, 1944, **154**, 581.
[152] Avineri-Shapiro and Hestrin, *Biochem. J.*, 1945, **39**, 167.
[153] Hehre, *Proc. Soc. Exp. Biol. N.Y.*, 1945, **58**, 219.
[154] Hehre, Genghof, and Neill, *J. Immunol.*, 1945, **51**, 5.

streptococci are able to synthesise dextran and levan simultaneously;[155] this versatility is displayed also by *Leuconostoc mesenteroides* NRRL B-512.[156]

In further studies of the levan sucrase of *B. subtilis*, Kohanyi and Dedonder[156a] have shown by paper chromatography that oligosaccharides are present at intermediate stages in the synthesis, while Doudoroff and O'Neal[157] have confirmed earlier observations[149, 150] that the reversibility of equation (xviii) cannot be demonstrated by treating a solution containing levan and glucose with the enzyme, because, at equilibrium, the forward reaction is highly favoured. The reverse reaction does proceed, however, if the equilibrium is disturbed by yeast invertase, which hydrolyses the sucrose as it is formed.[157]

Two important aspects of the enzymic synthesis require further study. First, a comprehensive investigation, by *chemical* methods, of the structure of the polysaccharide product is desirable, so that it can be shown whether the principal glycosidic linkages do, in fact, involve positions 2 and 6, and whether the molecules are unbranched, as they should be if levan sucrase is a single enzyme. Secondly, it has not yet been possible to demonstrate that primer molecules (*i.e.*, receptors of the transferred fructose units) are necessary to initiate the synthesis. It may well be that traces of levan in the enzyme fulfil this function (a possibility which was considered unlikely by Hestrin and Avineri-Shapiro,[150] since an enzyme prepared from cells grown on glucose was still active without the addition of levan), or, alternatively, that the substrate, sucrose, is itself a primer. Support for the latter hypothesis is to be found in the recent work of Palmer,[158] who has shown that the levan of *B. subtilis* contains a trace of glucose, which is most probably a part of the levan molecule and not of an associated impurity.

Synthesis of Fructosans of the Inulin Class

Although the enzymic synthesis of inulin has not yet been achieved *in vitro*, recent observations by several groups of workers seem to herald an early accomplishment of this aim. In a reinvestigation of the structure of the inulin of dahlia tubers, Hirst, McGilvray, and Percival[159] confirmed earlier reports of the presence of glucose residues (*ca.* 6%). Hydrolysis of the trimethyl ether of the polysaccharide yielded 1 : 3 : 4 : 6-tetra-*O*-methyl- and 3 : 4 : 6-tri-*O*-methyl-fructofuranose (3·2 and 91%), together with 2 : 3 : 4 : 6-tetra-*O*-methylglucopyranose (2·2%) and a mixture of tri-*O*-methylglucoses (3·2%). The high proportion of tetra- to tri-*O*-methylglucose suggested that the glucose residues were an integral part of the inulin molecule, and did not arise from an associated polyglucosan. Since no di-*O*-methyl sugars were encountered, a branched structure was excluded. On these grounds, it was concluded[159] that a possible structure for the inulin molecule is one in which a chain of *ca.* 35 fructofuranose units (linked through positions 1 and 2) is joined through the potential reducing group

[155] Hehre and Neill, *J. Exp. Med.*, 1946, **83**, 147.
[156] Jeanes, Wilham, and Miers, *J. Biol. Chem.*, 1948, **176**, 603.
[156a] *Compt. rend.*, 1951, **233**, 1142.
[157] *J. Biol. Chem.*, 1945, **159**, 585.
[158] *Biochem. J.*, 1951, **48**, 389.
[159] *J.*, 1950, 1297.

(by a sucrose-type linkage) to glucose, there being a second glucose residue (linked through positions 1 and 3) at some undetermined position within the fructose chain, as follows :

$$\text{Fru 2—[1 Fru 2]}_x\text{—3 Glu 1—[1 Fru 2]}_{35-x}\text{—1 Glu}$$

It is possible, of course, that the tri-*O*-methyl- but not the tetra-*O*-methyl-glucose arose from an associated polyglucosan, in which case the non-terminal glucose residue would be omitted. A similar structure has been suggested by Bacon and Edelman,[160] after a study of the oligosaccharides present in extracts of tubers, roots, and stems of the Jerusalem artichoke (an alternative source of inulin). They found, by paper chromatography, a series of oligosaccharides, each containing both glucose and fructose components ; sucrose was the lowest member and the others contained progressively higher fructose : glucose ratios. A related (possibly identical) series of oligosaccharides can be extracted from barley leaves.[161]

Having observed that artichoke tubers contain an enzyme (or enzymes) capable of producing the trisaccharide of the above series from mixtures of sucrose and inulin, but to a markedly smaller degree from either substrate alone, Bacon and Edelman [162] examined closely the course of the " hydrolysis " of sucrose to glucose and fructose, catalysed by yeast invertase. The same problem was studied simultaneously by Blanchard and Albon.[163] It was found that at least three saccharides, with R_F values less than that of sucrose, were formed during the initial stages of the reaction and disappeared later ; all of these saccharides were shown, by chromatographic procedures, to contain both glucose and fructose units.[162] When separated and purified, the triose fraction possessed two fructose residues and one of glucose ; since it was devoid of reducing properties it was believed to be a fructosyl-sucrose.[163] The oligosaccharides were produced with different concentrations of sucrose up to 55% and at any pH at which the invertase was active ; [163] the reaction was not modified by inorganic phosphate.[162]

It seems that yeast invertase functions by transferring fructose residues from sucrose to any carbohydrate receptor molecule present in the reaction mixture, and also to water,[164] probably *via* an intermediate fructose-2–enzyme complex,[165] thus [cf. equation (i)] :

$$\left.\begin{array}{lcl} (a)\ \text{Fru—Glu} + \text{E} & \rightleftharpoons & \text{Fru—E} + \text{Glu} \\ (b)\ \text{Fru—E} + \text{Fru—Glu} & \rightleftharpoons & \text{Fru—Fru—Glu} + \text{E} \\ (c)\ \text{Fru—E} + \text{Fru—Fru—Glu} & \rightleftharpoons & \text{Fru—Fru—Fru—Glu} + \text{E, etc.} \\ (d)\ \text{Fru—E} + \text{H}_2\text{O} & \xrightarrow[\text{slow}]{} & \text{Fru} + \text{E} \end{array}\right\} . \qquad \text{(xx)}$$

Such a mechanism explains the appearance of oligosaccharides during the early stages of the reaction, and attributes their subsequent degradation to the non-reversibility of reaction (*d*). It would be interesting to know whether a continuous removal of glucose (*e.g.*, by oxidation with notatin)

[160] *Biochem. J.*, 1951, **48**, 114.
[161] Porter and Edelman, *ibid.*, 1952, **50**, xxxiii.
[162] *Arch. Biochem.*, 1950, **28**, 467. [163] *Ibid.*, 1950, **29**, 220.
[164] Bealing and Bacon, *Biochem. J.*, 1951, **49**, lxxv.
[165] Fischer, Kohtès, and Fellig, *Helv. Chim. Acta*, 1951, **34**, 1132.

would lead to the synthesis of products of higher molecular weight. A claim by Aronoff [166] that the earlier workers were wrong in attributing oligosaccharide-synthesising activity to the invertase, rather than to an enzymic contaminant, has been disputed by White.[167] Recent observations by White and Secor [168] suggest that equation (xx) may be an over-simplification, because these authors found a second disaccharide, a second trisaccharide, and another oligosaccharide on chromatograms of the products of yeast invertase action on sucrose. The disaccharide (R_F < sucrose) was a *reducing* sugar containing a fructose and a glucose unit, but was not turanose (3-*O*-α-glucopyranosylfructopyranose). Both trisaccharide components contained two fructose residues and one of glucose. These observations seem to parallel those of Stodola *et al.*[126] on dextran sucrase (p. 72).

In addition to the above studies on yeast invertase, it has been shown that the invertase of *Aspergillus oryzæ* has a similar ability to transfer fructose residues.[137, 164, 169] By the time that 80% of the sucrose has disappeared, only 7·5% of the fructose units and 42·5% of the glucose units are present as the free sugars, the remainder being in the form of non-reducing oligosaccharides, which, however, *differ chromatographically from those produced with yeast invertase.*[164] On prolonged incubation, the oligosaccharides are destroyed, giving only glucose and fructose.[164] In conformity with equation (xx) the rate of oligosaccharide synthesis is reduced in the presence of glucose, but that of fructose formation is not.[164] The mould enzyme appears to differ from yeast invertase also in its ability to utilize raffinose as a substrate for the synthesis of higher saccharides.[162, 169] Pazur [169] has reported that it disproportionates raffinose into a tetrasaccharide (fructosylraffinose) of unknown structure and the disaccharide melibiose, as follows [cf. equation (i)] :

$$\left.\begin{array}{l}\text{Gal 1—6 Glu 1—2 Fru} + \text{Gal 1—6 Glu 1—2 Fru}\\ \qquad\qquad\qquad\qquad\quad \Uparrow\Downarrow\\ \text{Gal 1—6 Glu} + \text{Fru 2—(Gal 1—6 Glu 1—2 Fru)}\end{array}\right\} \quad . \quad . \quad \text{(xxi)}$$

In addition, he demonstrated, with the aid of ^{14}C-sucrose (labelled in both the glucose and the fructose portion), that the enzyme, acting on a mixture of sucrose and raffinose, transfers a fructose residue from the disaccharide to the trisaccharide.[169] As evidence that a single enzyme from the mould is responsible for the " hydrolytic " and " transfer " reactions, Bealing and Bacon [164] have shown that preparations from different species, from mycelia of different ages, and from crushed spores, give rise to quantitatively similar mixtures of free sugars and oligosaccharides, during their action on sucrose solutions of the same concentration.

Thus the enzymic synthesis of higher saccharides by invertase preparations has reached a most interesting stage of development, there being several important outstanding problems. It is imperative that, in future studies, high priority should be given to proof of the types of linkages present in the saccharides, because it is only by this means that the current assump-

[166] *Arch. Biochem. Biophys.*, 1951, **34**, 484. [167] *Ibid.*, 1952, **39**, 238.
[168] *Ibid.*, 1952, **36**, 490. [169] Pazur, *Fed. Proc.*, 1952, **11**, 267.

tion that these substances are in fact precursors of inulin can be verified. It may well be, for example, that the oligosaccharides produced by invertases of yeast and *Aspergillus oryzæ* have different R_F values because the fructosidic linkages are of the inulin type in one case and of the levan type in the other. Another complexity is introduced by the fact that sucrose, in which each of the sugar units is linked through its reducing group, is both a fructofuranoside and a glucopyranoside. Consequently invertases are of two types, fructo- and gluco-invertases. Since those mentioned above transfer fructose residues, they must belong to the first class ; members of the second class would transfer glucose units, thus :

$$\left.\begin{array}{rcl} \text{Glu 1—2 Fru} + \text{E} & \rightleftharpoons & \text{Glu—E} + \text{Fru} \\ \text{Glu—E} + \text{Glu 1—2 Fru} & \rightleftharpoons & \text{Glu—Glu 1—2 Fru} + \text{E} \\ \text{Glu—E} + \text{Glu—Glu 1—2 Fru} & \rightleftharpoons & \text{Glu—Glu—Glu 1—2 Fru} + \text{E} \\ \text{Glu—E} + \text{H}_2\text{O} & \longrightarrow & \text{Glu} + \text{E} \end{array}\right\} \quad \text{(xxii)}$$

Indeed, White [167] has already mentioned briefly that the gluco-invertase of honey converts sucrose into a series of glucosaccharides. This poses yet another problem : does the honey invertase differ from amylosucrase (see p. 69) or dextran sucrase (see p. 70), or both ?

General Summary

During the 15 years or so which have elapsed since studies of starch first began to yield information concerning the mechanisms of polysaccharide syntheses, great advances have been made. The present rapid rate of the accumulation of data can be attributed in large measure to the advent of paper chromatography, but, although it is undoubtedly extremely useful, this new weapon in the chemist's armoury must always supplement, and never replace entirely, the older techniques involving the isolation, purification, crystallisation, and careful characterisation of products.

Extensive as current knowledge is, it is still possible, as the foregoing pages testify, to relate all known authentic cases of the enzymic syntheses of polysaccharides to one fundamental reaction, in which, in the words of Bell,[170] " the energy associated with a pre-formed glycosidic link is used to form a new link by exchange of the originally substituting radical with a new one " [cf. equation (i)]. Attention was drawn to this fact by Doudoroff, Barker, and Hassid,[171] who suggested that an enzyme catalysing such a reaction should be termed a " transglycosidase ". It now seems to be generally accepted that each step in polysaccharide synthesis does not necessarily involve the direct exchange of the glycosidic link in the product for that in the substrate, but that the reaction may proceed *via* an enzyme glycoside (for a full discussion of this point, see Gottschalk [172]), and so equation (i) could be expanded to the following (E = enzyme residue) :

$$\left.\begin{array}{rcl} \text{G}_t\text{—O—X} + \text{Enzyme} & \rightleftharpoons & \text{G}_t\text{—E} + \text{HOX} \\ \text{G}_t\text{—E} + \text{H—O—G}_r & \rightleftharpoons & \text{G}_t\text{—O—G}_r + \text{Enzyme} \end{array}\right\} \quad \text{(xxiii)}$$

[170] *Ann. Reports*, 1947, **44**, 217.
[171] *J. Biol. Chem.*, 1947, **168**, 725.
[172] *Adv. Carbohydrate Chem.*, 1950, **5**, 49.

Now it has been shown,[173] with the aid of isotopically labelled oxygen, that muscle phosphorylase and sucrose phosphorylase (glucose-1 phosphate + fructose $\rightleftharpoons$ sucrose + phosphate) cleave glucose-1 phosphate between C and O, and not between O and P, so that in these cases the general substrate G_tOX is split at the G_t–O bond. This means that the glucose residue is transferred as the glucosyl group ($C_6H_{11}O_5$) and not as the glucosidyl-group ($C_6H_{11}O_6$), and it was for this reason that Hehre [108] suggested that "transglycosidases" should henceforth be known as "transglycosylases". It is not yet possible to decide whether this change in the terminology is justified for enzymes which utilise disaccharides as substrates.

An enzyme usually displays a high measure of specificity in its choice of substrate (G_tOX); so stringent are the structural and configurational requirements that frequently only one substance is known which will serve as a substrate for a given enzyme. Sometimes, however, higher homologues of the substrate are acceptable, and in such cases the additional sugar units may remain in the transferred residue (G_t) (*e.g.*, in the formation of *cyclo*amyloses), or become part of the rejected molecule (HOX) (*e.g.*, invertase on raffinose). Other cases in which G_t contains more than one sugar residue are the conversions of amylose into amylopectin and glycogen (Q-enzyme, Cori's branching factor). However, a given substrate may be utilised by several different enzymes; for example, higher saccharides are formed from glucose-1 phosphate by phosphorylase and sucrose phosphorylase, and from sucrose by amylosucrase, dextran sucrase, levan sucrase, and "invertases".

The receptor (HOG_r) must usually conform to a certain molecular type, but, within wide limits, may be of any molecular size; indeed, if molecular size were a critical factor, polysaccharide synthesis could never result, because the receptor molecule necessarily increases in length, progressively, as the synthesis proceeds. In certain cases (cf. the action of amylomaltase on maltose), HOG_r may be a second molecule of the substrate (G_tOX), but, where this is not permissible, oligosaccharides of the appropriate molecular type (primers) must be present before the synthesis can begin (cf. the phosphorylase-catalysed synthesis of amylose); to these primers the glycosyl units (G_t) are added successively at the non-reducing ends, and so the proportion of primer controls both the rate of synthesis and the chain length of the product. It has been postulated for some enzyme systems (*e.g.*, invertase, lactase) that water may function, essentially irreversibly, as the receptor (HOG_r), and thus the apparent dual roles of these enzymes as both transferases and hydrolases may be explained; it remains to be seen whether all enzymes hitherto recognised solely as carbohydrases fall into the same class.

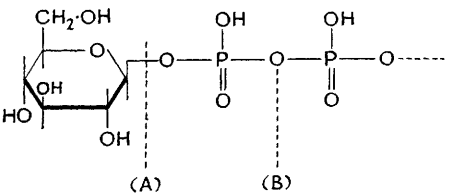
In this respect, "hydrolyses" of sugars by enzymes and by acids show a marked resemblance, for it has long been known that "reversion" (*i.e.*, the formation of a higher saccharide from a lower saccharide) may occur during acidic hydrolysis (cf. ref. 140*a*). Indeed, Pacsu and Mora [174] have

[173] Cohn, *J. Biol. Chem.*, 1949, **180**, 771.
[174] *J. Amer. Chem. Soc.*, 1950, **72**, 1045.

demonstrated that, under suitable conditions, polyglucoses containing some 40 units per molecule can be synthesised from glucose in this way. In the case of glucosylamine, the initial product of acidic hydrolysis is almost exclusively diglucosylamine.[175]

Another point which is clear from a study of all those enzyme reactions so far discussed, on which the necessary evidence is available, is that the transferred residue (G_t) retains its initial ring size and anomeric link in the polymeric product; thus an α-pyranoside always furnishes an α-linked pyranose polymer. It does not necessarily follow that the intermediate sugar–enzyme complex possesses a glycosidic link of the same anomeric type; indeed the conversion of G_tOX into G_t–E, and of G_t–E into G_tOG_r, could easily lead to Walden inversion, in which case G_tOX, G_tE, and G_tOG_r would be alternately α- and β-glycosides. Can it be that a small degree of racemisation during one of these transfers is responsible for the small percentage of β-linkages observed recently [176] in amylose? If so, these anomalous links should be found at the non-reducing chain-ends, because once a glucose residue became attached in this fashion, it would not serve as a receptor for further glucose units.

Although the major portion of published studies on the enzymic synthesis of polysaccharides is concerned with glucosans, in no case, as far as we are aware, has a β-linked polyglucose been synthesised *in vitro*. Since such a synthesis would almost certainly require a β-glucoside (*e.g.*, *iso*sucrose, cellobiose, β-D-glucose-1 phosphate) as the substrate, it is interesting that Fitting and Doudoroff [177] have reported recently an enzymic synthesis of β-D-glucose-1 phosphate (and glucose), from maltose and mineral phosphate, by means of an extract from *Neisseria meningitidis*. This phosphorylation is important in a second respect, because it is exceptional in that a change of the anomeric link from α to β is involved. A possible explanation of this unusual feature is that, in the intermediate glucose–enzyme complex, a β-glucosyl group is attached to the enzyme, or co-enzyme, at a pyrophosphate grouping, as follows:



and that the second stage of the reaction entails scission at (B) rather than (A).

Finally, the energy changes during transglycosylation must be considered. Hehre [108] has emphasised that polysaccharide synthesis is favoured by an exothermic exchange of glycosidic bonds; thus, since *iso*maltose

[175] Bayly, Bourne, and Stacey, *Nature*, 1952, **169**, 876.
[176] Peat, Thomas, and Whelan, *J.*, 1952, 722.
[177] *Fed. Proc.*, 1952, **11**, 212.

(1 : 6-α-link) is more stable to acid than is maltose (1 : 4-α-link),[178] the synthesis of a 1 : 6-α-glucosan from a 1 : 4-α-linked substrate should be favoured, and this is confirmed experimentally in the amylose → amylopectin, dextrin → dextran, and maltose → panose conversions. However, the energy-rich links of glucose-1 phosphate and sucrose make these substances energetically suitable as substrates for the synthesis of any glucosan. In the latter case, the rejected fructose unit is liberated in the furanose form and then rapidly assumes the more stable pyranose structure (cf. Isbell and Pigman [179]); consequently, syntheses of glucosans from sucrose are favoured to such an extent that the reverse reactions are difficult to demonstrate, unless the equilibrium is suitably disturbed by artificial means.

Although it is now possible to see how the syntheses of polysaccharides proceed according to a master plan, the subject is really only just emerging from its infancy, and for many years to come it will continue as a fascinating field of study. Problems of immediate interest are the enzymic syntheses of pentosans, β-glucosans, mannans, and other polysaccharides containing essentially a single sugar component; in these cases it will be necessary to consider how both the branched and the unbranched portions of the molecules arise. An explanation must be found, too, for the small percentage of anomalous linkages (*e.g.*, β-links in amylose, 1 : 3-links in amylopectin and in certain dextrans) which are now being revealed in such "simple" polysaccharides. Then attention must be paid to the biogenesis of polysaccharides carrying substituents (*e.g.*, chitin, fucoidin), and finally the challenge of the gums and mucilages must be accepted. Will it be a decade or a century before the chemist will be able to treat samples of D-galactose, D-glucuronic acid, L-arabinose, and L-rhamnose, in a predetermined sequence, with suitable specimens from his stock of crystalline enzymes and synthesise at will a sample of gum arabic?

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[178] Wolfrom, Lassettre, and O'Neill, *J. Amer. Chem. Soc.*, 1951, **73**, 595.
[179] *J. Res. Nat. Bur. Stand.*, 1938, **20**, 773.