561. *The Structure of the Starch-type Polysaccharide Synthesised from Sucrose by* Neisseria perflava.

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The polysaccharide synthesised from sucrose by cultures of *Neisseria perflava* (strain II—1) has properties intermediate between those of amylopectin and glycogen, approximating more closely to those of the latter. Its average chain length is 11—12 glucose units.

In 1946 Hehre and Hamilton (*J. Biol. Chem.*, 1946, **166**, 777) reported that *Neisseria perflava*, a Gram-negative coccus inhabiting the throats of healthy people, synthesises from sucrose, and to a smaller extent from glucose-1 phosphate, a polysaccharide of the amylopectin–glycogen class. It was shown subsequently that the washed cells of this organism (Hehre and Hamilton, *J. Bact.*, 1948, **55**, 197) and a cell-free enzyme system derived therefrom (Hehre, Hamilton, and Carlson, *J. Biol. Chem.*, 1949, **177**, 267) also catalyse the formation of an iodine-staining polysaccharide from these substrates. The American workers furnished strong evidence that *Neisseria perflava* utilises two enzymes, a phosphorylase and an amylosucrase, for the synthesis of 1 : 4-linked chains of α-D-glucose units.

Hehre, Hamilton, and Carlson (*loc. cit.*) observed that, whereas the polysaccharide produced by growing cultures is similar to maize amylopectin, that synthesised by the cell-free enzyme system more closely resembles glycogen. Dr. E. J. Hehre of the Cornell Medical School, New York, drew our attention to this fact and, at his suggestion, we have examined a polysaccharide isolated by him from *Neisseria perflava* cultures of a different strain (II—1) from that employed in the work mentioned above (19—34).

The bacterial polysaccharide was obtained free from proteins and other nitrogenous compounds and had an ash content of 1·5%. It dissolved slowly in cold water and rapidly in hot water to give an opalescent solution, which was stained reddish-purple with small amounts of iodine and red with larger amounts. Its reducing power, as measured by cuprimetric titration, was only 0·1% of that of glucose. The polysaccharide was not stained at 105° by ammoniacal silver nitrate or by an acid solution of resorcinol, and thus was virtually free from reducing aldoses and ketoses and also from acid-labile ketose sugars such as sucrose. Chromatographic analysis of a hydrolysate, obtained by heating the polysaccharide at 100° for 5 hours with 2N-sulphuric acid, revealed a single aldose component; this had an R_F value identical with that of glucose. Since no ketose was detected, the polysaccharide contained none of the fructose moiety of the sucrose substrate. The reducing power of such a hydrolysate corresponded to a 93—94% conversion into glucose.

Treatment of an aqueous solution of the bacterial polysaccharide with thymol under conditions which are favourable for the fractionation of potato and other starches (Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687) did not afford an insoluble complex. The blue value of the polysaccharide (0·09) was closely related to that of waxy maize starch (0·10; Bourne and Peat, *J.*, 1949, 5); it was intermediate between the blue values of guinea-pig liver glycogen (0·01) and of the purest samples of potato amylopectin prepared in these laboratories (0·14—0·18). Clearly the bacterial polysaccharide could have contained no more than a trace of amylose (B.V., 1·2—1·4). It will be recalled that Hehre, Hamilton, and Carlson (*loc. cit.*) showed that the polysaccharide synthesised by cultures of the 19—34 strain of *Neisseria perflava* likewise had a blue value intermediate between the blue values of glycogen and amylopectin and gave no precipitate when treated in aqueous solution with "Pentosol" (a commercial mixture of primary amyl alcohols) or butanol.

More detailed information regarding the structure of the bacterial polysaccharide (strain II—1) was obtained (*a*) by β-amylolysis, and (*b*) by methylation and end-group assay. The

limiting conversion into maltose with soya-bean β-amylase was 55—59% [cf. 57—59% for the polysaccharide from cultures of the 19—34 strain (Hehre, Hamilton, and Carlson, *loc. cit.*)]; the same enzyme sample was known to give 56% maltose from potato amylopectin (B.V., 0·21). Glycogens are usually regarded as having a somewhat lower conversion limit than this (Hopkins, Cope, and Green, *J. Inst. Brewing*, 1933, **39**, 487; Morris, *J. Biol. Chem.*, 1944, **154**, 503; Jeanloz, *Helv. Chim. Acta*, 1944, **27**, 1501; Hehre, Hamilton, and Carlson, *loc. cit.*), but Meyer and Jeanloz (*Helv. Chim. Acta*, 1943, **26**, 1784) have suggested that a low conversion may sometimes be caused by the inability of β-amylase to penetrate the more complex regions of the highly-branched glycogen structure. The limit dextrin produced by β-amylolysis of the bacterial polysaccharide, like that prepared from glycogen by Meyer and Fuld (*Helv. Chim. Acta*, 1941, **24**, 375), was stained faintly brown by iodine, in contrast to the relatively intense reddish-purple stain given by the limit dextrin derived from potato amylopectin. The blue value of the bacterial dextrin was *ca.* 0·01, whereas the potato dextrin has B.V., 0·15—0·20 (Bourne, Haworth, Macey, and Peat, *J.*, 1948, 924; Bourne, Sitch, and Peat, *J.*, 1949, 1448).

A hydrolysate of the methylated bacterial polysaccharide (OMe, 45·1%) gave, on a filter paper chromatogram, three spots, having R_F values identical with those of 2 : 3-dimethyl, 2 : 3 : 6-trimethyl, and 2 : 3 : 4 : 6-tetramethyl glucose. A quantitative assay on filter paper, with a technique which was essentially that described by Hirst, Hough, and Jones (*J.*, 1949, 928), showed the average chain length of the polysaccharide ether to be 11—12 glucose units, a value which agrees closely with the reported chain lengths of most glycogens (cf. Halsall, Hirst, and Jones, *J.*, 1947, 1399). The same method gave an average chain length of 20—21 glucose units for methylated potato amylopectin (Barker, Bourne, and Wilkinson, *J.*, 1950, in the press).

Thus the polysaccharides synthesised from sucrose by cultures of the 19—34 and the II—1 strain of *Neisseria perflava* appear to be closely related; they have properties intermediate between those usually accepted for glycogen and amylopectin. The balance of the above evidence tends to favour a glycogen structure for the latter bacterial polysaccharide, but we are of the opinion that there is no clear-cut distinction in nature between glycogen and amylopectin, since these two polysaccharides are synthesised by closely similar processes (Cori, Swanson, and Cori, *Fed. Proc.*, 1945, **4**, 234; Barker, Bourne, Wilkinson, and Peat, *J.*, 1950, 93). It is already known, for example, that some samples of rabbit liver glycogen have an average chain length of 18 glucose units (Haworth, Hirst, and Isherwood, *J.*, 1937, 577; Halsall, Hirst, and Jones, *loc. cit.*) and that a fraction of the polysaccharide from the Golden Bantam variety of sweet corn has an average chain length of 11 glucose units (Dvonch and Whistler, *J. Biol. Chem.*, 1949, **181**, 889). The different properties reported by Hehre and his colleagues (see above) for the polysaccharides synthesised by *Neisseria perflava* cultures and by the cell-free enzyme system may well have been caused by the preferential separation or even destruction, during the rupturing of the cells, of the enzymes responsible for the synthesis of 1 : 4-links relative to those which catalyse the formation of 1 : 6-links.

EXPERIMENTAL.

Isolation of the Bacterial Polysaccharide.—The polysaccharide (2·5 g.) was kindly provided by Dr. E. J. Hehre, by whom it had been isolated from the fluids of a sucrose-broth culture of *Neisseria perflava* (strain II—1); it had $[\alpha]_D^{17}$ +178° (*c*, 0·1 in 0·5N-sodium hydroxide). The method of preparation was similar to that employed by Hehre, Hamilton, and Carlson (*loc. cit.*) for the isolation of the polysaccharide synthesised by the 19—34 strain of *Neisseria perflava*.

Purity of the Polysaccharide.—(a) *Ash content.* Heated to constant weight in a micro-muffle furnace, the polysaccharide gave 1·5% of ash.

(b) *Nitrogenous impurities.* Negative reactions were obtained when the polysaccharide was tested for nitrogenous impurities by fusion with sodium, and for proteins by treatment with Millon's mercuric nitrite reagent.

(c) *Reducing sugars.* The reducing power of a solution (5 c.c.), containing the polysaccharide (30·6 mg.) and glucose (0·50 mg.), was measured by means of the Shaffer–Hartmann copper reagent (*J. Biol. Chem.*, 1921, **45**, 377), calibrated against glucose. The reducing power of the polysaccharide, calculated by difference, was 0·1%, in terms of glucose.

Spots of a solution (2%) of the polysaccharide, applied to filter papers, were sprayed (i) with an ammoniacal solution of silver nitrate, and (ii) with an acid solution of resorcinol. Neither reagent gave a stain when heated to 105°, showing the virtual absence of reducing sugars of both the aldose and the ketose series, and of acid-labile sugars, such as sucrose, which yield a ketose on hydrolysis.

(d) *Reducing sugars liberated during acidic hydrolysis.* (i) The polysaccharide (40 mg.) was heated at 100° for 5 hours with 2N-sulphuric acid (1 c.c.) in a sealed tube. The hydrolysate and water washings (1 c.c.) were neutralised with barium carbonate and centrifuged. Spots of the supernatant solution and of three reference solutions (2%), containing glucose, fructose, and sucrose, respectively, were applied to a sheet of filter paper (Whatman No. 1) and irrigated for 24 hours with aqueous butanol, as described by

Partridge (*Nature*, 1946, **158**, 270). After drying, the sheet was developed with ammoniacal silver nitrate. The reference solutions of glucose and fructose and the hydrolysate of the polysaccharide each gave one spot, the R_F value of the unknown sugar being indistinguishable from that of glucose. A similar sheet, developed with acid resorcinol, revealed the reference fructose and sucrose spots, but no spot from the hydrolysate of the polysaccharide.

(ii) Duplicate samples (*ca.* 25 mg.) of the dry polysaccharide were hydrolysed for 5 hours with 7% sulphuric acid and analysed by cuprimetric titration, as described by Bourne, Donnison, Haworth, and Peat (*loc. cit.*). The reducing powers of the neutral hydrolysates corresponded to conversions into glucose of 93 and 94%, respectively.

Treatment of the Polysaccharide with Thymol.—Powdered thymol (0·2 g.) was added to an aqueous solution (30 c.c.) of the bacterial polysaccharide, and the suspension was kept at 30° for 3 days. No insoluble polysaccharide–thymol complex was formed.

Iodine Stain.—The polysaccharide was stained with iodine under the standard conditions for the determination of blue value (B.V.) described by Bourne, Haworth, Macey, and Peat (*loc. cit.*). The final solution contained the polysaccharide (1 mg./100 c.c.), iodine (2 mg./100 c.c.), and potassium iodide (20 mg./100 c.c.). The absorption values (A.V.) of this solution are compared in the following table with those obtained when samples of potato amylopectin, prepared by the *cyclo*hexanol method (Bourne, Donnison, Haworth, and Peat, *loc. cit.*), and glycogen from guinea-pig liver are stained under similar conditions.

TABLE I.

Absorption values of polysaccharide–iodine solutions.

Polysaccharide.	A.V. at λ: 4300 A.	4700 A.	4900 A.	5200 A.	5500 A.	5800 A.	6000 A.	6800 A.
Potato amylopectin *	0·45	0·34	0·30	0·29	0·30	0·28	0·25	0·18
Neisseria perflava polysaccharide	0·52	0·37	0·34	0·31	0·26	0·21	0·13	0·09
Glycogen from guinea-pig liver †	0·38	0·21	0·14	0·08	0·04	0·02	0·01	0·01

* From a graph given by Bourne, Donnison, Haworth, and Peat (*loc. cit.*).
† Kindly supplied by Dr. F. Smith.

Treatment of the Polysaccharide with β-Amylase.—(a) *Conversion limit.* The bacterial polysaccharide was submitted to β-amylolysis under the conditions described by Bourne, Donnison, Haworth, and Peat (*loc. cit.*). The percentage conversion into maltose was 42 (30 minutes), 54 (180 minutes), and 55 (270 minutes).

(b) *Isolation of the limit dextrin.* A solution of the polysaccharide (260·9 mg.) in 0·5N-sodium hydroxide (20 c.c.) was neutralised with sulphuric acid and buffered at pH 4·7 with M-acetate buffer (pH, 7·0; 10 c.c.) and N-acetic acid (10 c.c.). After the addition of a 0·2% solution (30 c.c.) of soya-bean β-amylase (Bourne, Macey, and Peat, *J.*, 1945, 882), the solution was diluted with water to 100 c.c. and incubated at 28°. Estimations of reducing power by the Shaffer–Hartmann method (*loc. cit.*) indicated that the conversion limit was reached after 18 hours. Three hours later, the digest was inactivated by immersion in a boiling water-bath and the coagulated protein was removed by filtration. The reducing power of the filtrate corresponded to a 59% conversion into maltose. The solution was dialysed in Cellophane against frequent changes of distilled water for 3 days and then freeze-dried. The limit dextrin (75·5 mg.) represented 30% of the original polysaccharide (allowance being made for the digest samples which had been removed for analytical purposes).

The limit dextrin gave a brown iodine stain in high concentration, but gave no detectable stain under the conditions required for the determination of blue value (see above). A solution containing the dextrin (5 mg./100 c.c.), iodine (2 mg./100 c.c.), and potassium iodide (20 mg./100 c.c.) had the absorption values quoted in Table II.

TABLE II.

Absorption values of the dextrin–iodine solution.

Solution.	A.V. at λ: 4300 A.	4700 A.	4900 A.	5200 A.	5500 A.	5800 A.	6000 A.	6800 A.
Dextrin + I_2 + KI	0·43	0·27	0·21	0·14	0·11	0·07	0·03	0·02
I_2 + KI	0·35	0·19	0·11	0·06	0·03	0·02	0·01	0·01

Methylation of the Bacterial Polysaccharide.—The bacterial polysaccharide (0·963 g.) was methylated with sodium and methyl iodide in liquid-ammonia suspension at −70° (Freudenberg and Boppel, *Ber.*, 1938, **71**, 2505). In all 15 alternate additions of sodium and methyl iodide were made. The liquid ammonia was allowed to evaporate at room temperature, the final traces being removed at 100°/12 mm. The residue was dissolved in water and dialysed for three days against frequent changes of distilled water. The solution was concentrated by freeze-drying to a white solid, which was finally dried at 60° over phosphoric oxide in a vacuum. The methylated polysaccharide (0·808 g.) had OMe, 45·1% (the trimethyl ether of a polyglucose requires OMe, 45·6%).

Identification of the Methyl Sugars Obtained from the Methylated Polysaccharide.—The methylated polysaccharide (60 mg.) was heated for 9 hours with methanolic hydrogen chloride (3·6%, w/w; 1·2 c.c.) in a sealed tube immersed in a boiling water-bath. When cool, the tube was opened, 4% hydrochloric acid (6 c.c.) was added, and the glucoside mixture was hydrolysed at 100° for 5 hours. The mixed methyl

sugars were isolated by the method of Hirst, Hough, and Jones (*loc. cit.*) and redissolved in water (1 c.c.). In a similar manner, a hydrolysate was prepared from methylated potato amylopectin (OMe, 44·5%). A reference solution (3 c.c.), containing 2 : 3-dimethyl glucose (3·12 mg.), 2 : 3 : 6-trimethyl glucose (66·6 mg.), and 2 : 3 : 4 : 6-tetramethyl glucose (3·54 mg.), was also prepared.

Spots of the hydrolysates and of the reference solution were placed on filter paper (Whatman No. 1) and irrigated for 18 hours with a butanol solvent, as described by Hirst, Hough, and Jones (*loc. cit.*). An aniline hydrogen phthalate spray (Partridge, *Nature*, 1949, **164**, 443) revealed three spots, identical in each case.

End-Group Assay of the Methylated Polysaccharide.—The components of a hydrolysate of the methylated polysaccharide were separated by filter paper chromatography, eluted, and estimated by the hypoiodite method according to the procedure of Hirst, Hough, and Jones (*loc. cit.*) with minor modifications described by Barker, Bourne, and Wilkinson (*loc. cit.*). The results of five independent assays are shown in Table III.

TABLE III.

End-group assay of the methylated bacterial polysaccharide.

Assay number.	Molecular ratio (%) of components.		
	2 : 3-Dimethyl glucose.	2 : 3 : 6-Trimethyl glucose.	2: 3 : 4 : 6-Tetramethyl glucose.
I	6·5	83·7	9·8
II	4·5	86·2	9·3
III	6·0	85·5	8·9
IV	5·8	85·8	8·3
V	7·4	85·1	7·4
Average	6·0	85·3	8·7

This proportion of tetramethyl glucose corresponded to a chain length of 11—12 glucose units.

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