Cl_4, *c* 0.8), $[\alpha]^{23}D$ +4° (*glacial* HOAc, *c* 0.8), $[\alpha]^{21}D$ −32° ($SOCl_2$, *c* 0.6), $[\alpha]^{21}D$ −22° (dioxane, *c* 1).

Anal. Calcd.[25] for $C_{142}H_{187}O_{95}Cl$: C, 49.44; H, 5.46; Cl, 1.03; OAc, 43.63. Found: C, 48.53; H, 5.22; Cl, 1.03–1.60; OAc,[26] 43.07.

Decarboxylation of Reacetylated Reduced Oxidized Cellulose Acetate.—The product formed by the reaction of reacetylated reduced oxidized cellulose acetate with thionyl chloride was treated with silver oxide and bromine following the procedure used for the product obtained from the reaction of oxidized cellulose acetate with thionyl chloride; $[\alpha]^{15}D$ −12.3° ($C_2H_2Cl_4$, *c* 0.4), $[\alpha]^{17}D$ ±0.0° (*glacial* HOAc, *c* 0.6), $[\alpha]^{16}D$ −14° (dioxane, *c* 1), $[\alpha]^{24}D$ −13.5° ($SOCl_2$, *c* 1.7).

Anal. Calcd.[25] for $C_{141}H_{187}O_{94}Br$: C, 48.86; H, 5.44; Br, 2.31; OAc, 43.42; CO_2, 5.8 ml. of 0.1 *N* NaOH per g. Found: C, 48.47; H, 5.22; Br, 2.50; OAc,[26] 43.07; CO_2, 6.1 ml. of 0.1 *N* NaOH per g.

Reduction of Decarboxylated Reacetylated Reduced Oxidized Cellulose Acetate with Sodium Borohydride.—An amount of 5. g. of the decarboxylated reacetylated reduced oxidized cellulose acetate was dissolved in 500 ml. of dioxane (distilled from sodium). Sodium borohydride (2 g.) was added and the mixture stirred for 4 hours at 40–50°. Stirring was continued overnight at room temperature, at the end of which time a thick thixotropic gel had formed. The gel was broken up, filtered and the solid triturated with water (2 × 500 ml.), filtered, and washed with aqueous half-saturated sodium bicarbonate (2 × 100 ml.), water (2 × 500 ml.) and absolute ethanol (2 × 500 ml.). The polymer was dried overnight under a high vacuum at room temperature and then dissolved in 500 ml. of dioxane and precipitated by the addition of approximately two volumes of ether; yield approximately 5 g.; $[\alpha]^{18}D$ −20° ($C_2H_2Cl_4$, *c* 0.4), $[\alpha]^{20}D$ +5° (*glacial* HOAc, *c* 0.8), $[\alpha]^{21}D$ −21° ($SOCl_2$, *c* 0.4); $[\alpha]^{18}D$ −19° (dioxane, *c* 1).

Anal. Calcd.[25] for $C_{141}H_{188}O_{94}$: C, 49.99; H, 5.60; OAc, 44.44. Found: C, 49.85; H, 5.55; OAc,[26] 42.56.

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The Interaction of Bacterial Polyglucosans with Concanavalin-A

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The globulin, concanavalin-A, obtained from Jack Bean meal, which has been shown previously to give a precipitate with glycogen and not with the apparently closely related amylopectin, has been used to ascertain whether certain bacterial polyglucosans are related to glycogen or to amylopectin.

Glycogen and amylopectin have been reported to be similar in respect to properties and structures.[2–5] Some of the properties displayed by the polyglucosans obtained from *Neisseria perflava* appear to be intermediate between glycogen and amylopectin.[6,7] One preparation was found[3,13] by periodate oxidation and by methylation to have, like glycogen, an average repeating unit of about 12 residues whereas it behaved like amylopectin in giving a reddish-purple color with iodine and undergoing a 55–59% degradation upon treatment with β-amylase.

In view of the apparent relationship of the *Neisseria* polyglucosans to both glycogen and amylopectin it was decided to investigate their behavior when treated with concanavalin-A, a globulin from jackbean meal,[8–12] since it had been observed that this protein gives a precipitate with glycogen[9,11,12] but not with amylopectin.[12]

Certain of the enteric bacterial polyglucosans were likewise investigated as were various dextrans to ascertain whether they reacted with concanavalin-A.

Methods.—The preparation of concanavalin-A reagent from jackbean meal was described earlier.[11,12] The procedure for estimating "glycogen-values" is based on the absorbancy obtained upon treatment of one mg. of the material with the concanavalin-A reagent, in relation to the absorbancy obtained upon similar treatment of one mg. of a standard glycogen.[11,12]

α-Amylolysis was carried out by treating 0.2% solutions of the polysaccharides at 25° with 0.1 volume of diluted saliva (1:10) buffered to *p*H 7.0 with phosphate.

β-Amylolysis was performed at *p*H 4.5 and 25°, using 1 mg. of Wallerstein β-amylase of analytical purity with 100 mg. of polysaccharide in 50 ml. of 0.01 *M* acetate buffer solution.

Results and Discussion

It had been noted earlier[12] that neither amylopectin nor its β-limit dextrin were precipitated on treatment with concanavalin-A reagent, whereas all of the glycogens examined did so. Since *Neisseria perflava* cultures which contained sucrose were stated to produce amylopectin-like polysaccharides having in some respects properties intermediate between those of glycogen and amylopectin,[3,6] it appeared of interest to examine the *Neisseria* polysaccharides with concanavalin reagent. The results are shown in Table I.

Contrary to the results obtained with plant amylopectins, the *Neisseria* amylopectin-like sub-

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TABLE I

THE INTERACTION OF POLYGLUCOSANS WITH CONCANAVALIN-A

Preparation	Organism used	Apparent glucosan type	Absorbancy	Glycogen value
19–34	*N. perflava* (live culture)	Amylopectin	0.137	0.80
II-1	*N. perflava* (live culture)	Amylopectin	.125	.75
II-2	*N. perflava* (live culture)	Amylopectin	.125	.75
19-34	*N. perflava* (cell-free enzyme)	Glycogen	.170	1.00
4943[a]	*Acetobacter capsulatum*	Dextran	.099	0.60
F90A[a]	*Streptococcus* (group H)	Dextran	.000	.00
NRRL B-512[a]	*Leuconostoc mesenteroides*	Dextran	.006	.05
P(CL) 3-1	*Salmonella montevideo*[b]	Glycogen	.232	1.35
A12, KOH prep.	*Aerobacter aerogenes*[c]	Glycogen	.188	1.10
A12, sonic prep.	*A. aerogenes*[d]	Glycogen	.177	1.05

[a] Dissolved with the aid of alkali (*N* NaOH) and neutralized with *N* HCl. [b] Grown on 1% mannose–nutrient agar and isolated by extraction with KOH and ethanol precipitation. [c] Grown on 1% glucose–nutrient agar and isolated as in *b*. [d] As in *b*, but a sonic lysate.

stances all show significant reactions with the concanavalin reagent. Though the glycogen values of the substance were somewhat lower than are generally obtained with animal or plant glycogens, it will be noted from Table II that fractionation of one of the *Neisseria* polysaccharides (II-1) gave three fractions (I, 20%; II, 60% and III, 20%, respectively, of the material fractionated) with increasing glycogen values. It thus appears that these products may indeed be largely glycogen-like and that any amylopectin-like material is probably present to a smaller extent.

TABLE II

EFFECT OF FRACTIONATION[a] ON THE GLYCOGEN VALUES OF CERTAIN BACTERIAL GLUCOSANS

Preparation	Fraction 1 G. value	Fraction 2 G. value	Fraction 3 G. value
N. perflava II-1	0.70	0.95	1.05
A. capsulatum 4943	.75	.60	

[a] Fractionation was effected in the usual way by adding alcohol to an aqueous solution of the polysaccharide.

On the other hand, the cell-free system from *Neisseria perflava* cultures produces a glycogen-like substance when incubated with sucrose. The material obtained in this way from strain 19–34 had a glycogen value corresponding to those shown by normal human and rabbit liver glycogens.[12] Periodate oxidation indicated an average chain length of 11 units.[13] From these results it appears that both the cell-free and the cell-containing systems produce glycogen-like substances while any amylopectin-like material that is produced appears in cultures of the growing cells. The fact that the amylopectin-like preparation, II-1, was found[3] by methylation studies to have a chain length of 11–12 residues, further adds to the probability that glycogen is the main constituent. The possibility that the substance is homogeneous and represents a polysaccharide with properties intermediate between glycogen and amylopectin appears to be ruled out by the fractionation results (see Table II) which show that increasing glycogen values are obtained for successive fractions.

Reactions such as iodine coloration and extent of degradation by β-amylase are inadequate criteria for distinguishing between the two types of polysaccharides. Thus, while preparation II-1 showed β-amylolysis of 55–59%, values of 25–56% have been recorded[4,14] for various animal glycogens and a rabbit liver glycogen sample, reported to have an 18 unit branch length[15,16] was found to be degraded 53% by β-amylase. Similarly, the red-purple color which the amylopectin-like substances produce with iodine is much like that obtained with rabbit leg muscle glycogen.[12] The similarity in properties of glycogen and amylopectin makes it difficult to distinguish glycogen when admixed with amylopectin and it would not be surprising that a mixture of the two should be looked upon as being amylopectin-like. It would be interesting to investigate with concanavalin-A other glucosans with apparently overlapping properties such as the Floridean and Ulva starches described by Meeuse and Kreger[17] which give X-ray powder diagrams much like potato starch but have average chain lengths, as determined by periodate oxidation, similar to that of glycogen.

An interesting behavior of the *Neisseria* polyglucosans was encountered when these were treated with salivary α-amylase. Each of the polysaccharides failed to be degraded sufficiently during 5 hours incubation with diluted saliva buffered at *p*H 7.0 to diminish completely the reaction with the concanavalin reagent. The extent of degradation as reflected by reaction with concanavalin varies considerably for the different preparations. However, one of the preparations (II-1) was fractionated with ethanol and it was then found that the fractions obtained were degraded in 10 minutes by the salivary α-amylase to the extent that no concanavalin reaction was obtained. Similar results were noted with certain muscle glycogens.[12]

Another unusual reaction concerns the synthesis[18] of a dextran by an *Acetobacter capsulatum* enzyme system acting upon dextrins instead of sucrose which is the substrate for the formation of dextran by *Leuconostoc mesenteroides*. Equally unusual is the result obtained in the treatment of

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the *Acetobacter* dextran with concanavalin reagent. Thus, while the dextrans formed by *Leuconostoc mesenteroides* and *Streptococcus* group H, show no significant reaction with concanavalin, the polysaccharide formed by *Acetobacter capsulatum* shows a glycogen-value equivalent to 60% of that shown by normal human liver glycogen (see Table I).

Fractionation of the polyglucosan synthesized by *Acetobacter capsulatum* on a limited scale gave two fractions, one of which showed a small increase in the glycogen value (Table II). Unlike the *Neisseria* polysaccharide which gave rise to fractions that became less opalescent in aqueous solution as the glycogen value increased, the *Acetobacter* polysaccharide fraction which formed the more opalescent solution showed the larger glycogen value.

The above results indicate that the *Acetobacter* polysaccharide must differ in some respect from the other dextrans tested. The nature of this difference has yet to be ascertained. That the results may be due to the presence of glycogen-like material is possible but unlikely since periodate oxidation, in which the periodate consumption was 1.89 moles per anhydroglucose unit,[18] resulted in the formation of 0.82 mole of formic acid per anhydroglucose unit. Such results favor a dextran type of structure for the *Acetobacter* polyglucosan in which the ratio of 1,6-linkages to other types is 5:1. It is conceivable that the difference between the *Leuconostoc* dextran and the *Acetobacter* polyglucosan is due to the presence of linkages in the latter which are not of the 1,6-type. The formation of dextrans with varying types of linkages[19] may make it possible to test this hypothesis and information bearing on this point is now being sought.

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Reports on the isolation of glycogen-like substances from bacteria other than *Neisseria* have been few. Glycogens have been isolated from avian tubercle bacilli,[20] from *Bacillus megatherium*[21] and most recently from enteric bacteria.[22]

Glycogens from *Aerobacter aerogenes* and *Salmonella montevideo* (enteric bacteria) grown on nutrient agar containing 1% mannose for the *Salmonella* and 1% glucose for the *Aerobacter* cultures were examined by means of the concanavalin–polysaccharide reaction. The results which are shown in Table I indicate that the bacteria produced glycogens of the normal type, although the *Salmonella* glycogen gave a somewhat higher than average glycogen-value.

Treatment of the three preparations from the enteric bacteria with diluted saliva resulted in their rapid degradation to the point where they no longer reacted visibly with concanavalin reagent. It was interesting, however, that the *Salmonella* glycogen which was slightly impure as obtained from Dr. Levine was not degraded by β-amylase until it was further purified. All three of the purified glycogens underwent a 30–40% hydrolysis when treated with β-amylase, a result in agreement with the values shown by most animal glycogens.

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Anomeric 1-Dicyclohexylammonium Phosphate Esters of D-Glucopyranose, D-Galactopyranose, D-Xylopyranose and L-Arabinopyranose[1]

BY E. W. PUTMAN AND W. Z. HASSID

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Three new phosphorylated pentose derivatives, β-D-xylose 1-phosphate and the α- and β-forms of L-arabinose 1-phosphate have been obtained as crystalline cyclohexylammonium salts. In addition the cyclohexylammonium salts of the 1-phosphoric acid esters of α-D-xylose, α- and β-D-glucose and α- and β-D-galactose have been prepared. The cyclohexylammonium salts of these phosphorylated sugars may be purified readily by recrystallization, in contrast to the barium salts which are amorphous and usually contain anomeric impurities. Application of Hudson's isorotation rules to the α- and β-anomers of the four phosphorylated sugars shows the 2A values to be consistent with the first isorotation rule, the average value being 25,400. However, the 2B values are greater than those of the corresponding methyl glycosides by about 9,000 molecular rotation units.

Previous investigations revealed that mung bean seedlings contain uridyl pyrophosphorylases capable of catalyzing the reversible formation of uridine diphosphate D-glucose from uridine triphosphate and α-D-glucose 1-phosphate, as well as the formation of uridine diphosphate D-xylose from uridine triphosphate and α-D-xylose 1-phosphate.[2] For continuation of these studies pure α- and β-forms of 1-phosphate esters of D-galactose, D-glucose, D-xylose and L-arabinose were required.

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