Biochemistry of the Sphingolipids. XVII. The Nature of the Oligosaccharide Component of Phytoglycolipid*

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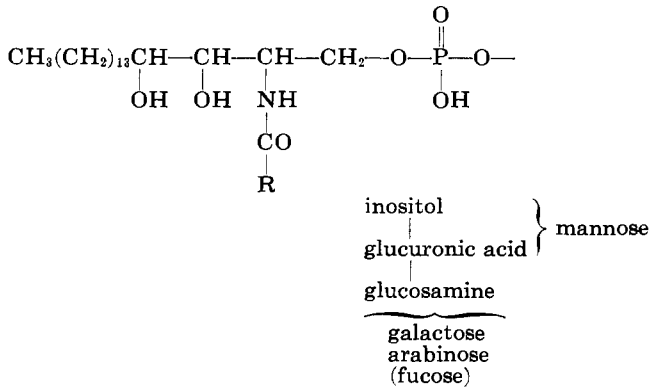
The oligosaccharide fraction obtained by hydrolysis of phytoglycolipid from corn has been separated on Dowex-2(HCO_3^-) into a series of oligosaccharides of progressively increasing molecular weight. The first number of the series is the trisaccharide, glucosaminido-glucuronido-inositol. This trisaccharide is obtained by mild acid hydrolysis of all the higher oligosaccharides and accounts essentially quantitatively for their glucosamine and glucuronic acid content. The tetrasaccharide fraction was characterized by preparation of crystalline derivatives and was shown to contain only mannose (in addition to trisaccharide). The higher oligosaccharide fractions contained, in addition, varying amounts of galactose, arabinose, and fucose. The penta-, hexa-, hepta-, and octasaccharide fractions were in each case mixtures from which no single constituent was separated.

In previous publications (Carter *et al.*, 1958b, 1962, 1964) evidence has been presented for the following partial structure for phytoglycolipid, a complex glycolipid found in corn, soybean, flax, and other seeds.



The oligosaccharide moieties of corn and soybean phytoglycolipid have been obtained by alkaline hydrolysis of the corresponding lipids. Each of the oligosaccharides gave similar analytical data approximating those required for a hexasaccharide containing inositol, glucuronic acid, glucosamine, galactose, arabinose, and mannose, and each on mild acid hydrolysis gave an excellent yield of a crystalline trisaccharide, glucosaminido - glucuronido - inositol. This trisaccharide is readily obtained as a characteristic degradation product of each of the phytoglycolipid samples studied and accounts for essentially all of the glucosamine and glucuronic acid content.

In the course of these studies flax phytoglycolipid was prepared and discovered to contain fucose in addition to the other carbohydrate components. Subsequent investigation disclosed the presence of small amounts of fucose in soybean and corn phytoglycolipid. These findings prompted a more thorough study of the composition of the oligosaccharide components of phytoglycolipid. The present paper reports results with the oligosaccharide from corn.

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The first indication of the heterogeneity of the oligosaccharide was provided by paper chromatography. The free oligosaccharide gave an elongated spot (R_F 0.02–0.26) in 1-butanol-pyridine-water (6:4:5). (Under these same conditions glucosaminido-glucuronido-inositol gave an R_F of 0.24, glucosylinositol an R_F of 0.26, and inositol an R_F of 0.35). However, all efforts to obtain separate discrete spots from the oligosaccharide failed. Proof of the heterogeneity of the oligosaccharide fraction was obtained finally in attempts to purify it on carbon-Celite columns by the method of Whistler and Durso (1950). Development of the column with increasing concentrations of aqueous ethanol gave a series of fractions. The early peaks contained, in addition to trisaccharide, mannose (major component) and galactose (minor component), but arabinose and fucose were absent. In later fractions galactose was the major component with mannose, arabinose, and fucose present as minor constituents. A similar fractionation using a mixture of free and phosphorylated oligosaccharide gave similar distribution of sample and sugar composition.

Although some fractionation of the oligosaccharide mixture was effected on carbon columns, a clean separation of components was not achieved. Attention was therefore turned to the use of ion-exchange resins. Partial resolution was obtained over columns of Dowex-1 (OAc^-) with an acetic acid–water gradient elution. The results with Dowex-2 (HCO_3^-) resin were more promising and after some experimentation with resin mesh size, column size, and elution rates a procedure was developed which gave six peak fractions. Each fraction was characterized by analyses for the constituent sugars and by the isolation of glucosaminido-glucuronido-inositol from acid hydrolysates.

Fraction A, accounting for 9% of the original material, proved to be glucosaminido-glucuronido-inositol. The major peak, fraction B, contained mannose and a trace of galactose in addition to glucosamine, glucuronic acid, and inositol. The main component of fraction B moved as a tetrasaccharide on Sephadex G-25 and was completely devoid of galactose, arabinose, and fucose. The analytical data on the free amorphous tetrasaccharide and the crystalline *N*-acetyl derivative are in excellent agreement with those of a tetrasaccharide containing inositol, glucuronic acid, glucosamine, and mannose. The *N*-acetyl tetrasaccharide was further characterized by reduction of the carboxyl group to give a crystalline substance containing equimolar amounts of inositol, glucose, *N*-acetylglucosamine, and mannose.

The remaining fractions contained major amounts of galactose plus mannose, arabinose, and fucose. The nitrogen content and trisaccharide content decreased progressively from fraction B to fraction F. Analytical data and results of chromatography on paper and on Sephadex G-25 strongly indicate that the major components of fractions C, D, E, and F are, respectively, penta-, hexa-, hepta-, and octasaccharides. The carbohydrate composition of these fractions cannot be reconciled with the presence of a single component. It seems certain that these fractions are mixtures of various possible pentasaccharides, hexasaccharides, and so on.

It is concluded that the oligosaccharide mixture obtained by hydrolysis of corn phytoglycolipid has the following approximate composition:

Component		%
A	Glucosaminido-glucuronido-inositol	9
B	Tetrasaccharide ([mannosido]-glucosaminido-glucuronido-inositol)	41
C	Pentasaccharides	10
D	Hexasaccharides	10
E	Heptasaccharides	14
F	Octa and higher oligosaccharides	8

Phosphorylated oligosaccharide has a similar composition with the addition of the phosphate group. Elucidation of the structure of the tri- and tetrasaccharide and further characterization of the higher oligosaccharides will be reported in a subsequent publication.

Experimental

Analytical Procedures.—The nitrogen content of column fractions was determined by the Dumas method. All other nitrogen analyses were performed by micro-Kjeldahl procedures. Phosphorus determinations were made using a slight modification of the method of Harris and Popat (1954). "Long-chain-base" nitrogen was determined in the initial experiments by the procedure of McKibben and Taylor (1949). Later determinations were made using a slight modification of the method of Lauter and Trams (1962). Glucuronic acid was determined using an adaption (Bitter and Ewins, 1961) of the Dische carbazole test for hexuronic acids. Glucosamine content of fractions was calculated from the nitrogen content since it had been shown in previous work that essentially all of the nitrogen was present as glucosamine.

Mannose, galactose, and arabinose were separated by preparative chromatography and characterized both qualitatively and quantitatively by procedures described previously (Carter *et al.*, 1962, 1958a).

Paper Chromatography.—Method 1.—Triple-ascent chromatograms were run to a height of 20 cm using a 1-butanol-pyridine-water (6:4:5) solvent system. Papergrams were dried and developed by spraying with (a) 0.5% periodate followed by benzidine reagent, (b) aniline-phthalate reagent, or (c) 2% ninhydrin in pyridine.

Method 2.—Chromatograms were developed for 24 hours in an ethyl acetate–acetic acid–water (3:1:3, upper phase) solvent system using the descending technique. The dried chromatograms were sprayed with aniline-phthalate reagent and then heated at 100° for 5 minutes. The solvent system was freshly prepared and allowed to stand for 12 hours before use.

Preparation of Phytoglycolipid.—Samples of phytoglycolipid were prepared from corn phosphatide and from corn inositol lipid by the procedures previously described (Carter *et al.*, 1958a). Various preparations gave the following analytical data:

Phytoglycolipid from corn phosphatide: N, 1.94, 1.98; P, 2.08, 2.02; long-chain-base nitrogen, 0.69, 0.72; ash, 3.4, 2.5%.

Phytoglycolipid from corn inositol lipid: N, 1.59, 1.61; P, 2.04, 1.95; long-chain-base nitrogen, 0.83, 0.77; ash, 2.3, 3.8%.

Preparation of Oligosaccharide Fraction.—Barium hydroxide hydrolysis.—Purified phytoglycolipid (20 g) was refluxed with 1800 ml of saturated barium hydroxide solution for 8 hours. The resulting suspension was cooled in an ice bath and filtered. The filtrate was passed rapidly (100 ml/min) through a column (5 × 80 cm) of Amberlite IRC-50 (H^+) resin and the resin was washed thoroughly with water (7 liters). The combined filtrate was evaporated *in vacuo* below 35° to yield a mixture of phosphorylated and nonphosphorylated oligosaccharides (10.0 g). Found: N, 1.21; P, 0.63%.

The slight decomposition of the oligosaccharide mixture, after passage over the resin, was avoided in subsequent preparations by adjusting the *p*H of the eluate to 6.0 by addition of the minimum quantity of Dowex-2 (HCO_3^-) resin.

The original insoluble residue (13.0 g) from the hydrolysis was acidified with concentrated hydrochloric acid. The mixture was thoroughly extracted with chloroform, neutralized by addition of more barium nydroxide, and lyophilized to yield a cream powder (5.0 g). Found: N, 0.43; P, 1.06; long-chain-base nitrogen, 0.0, ash, 65%.

Lithium hydroxide hydrolysis.—Purified phytoglycolipid (20.0 g) was refluxed with 1 N lithium hydroxide solution (1500 ml) for 8 hours. The mixture was cooled in an ice bath and acidified to *p*H 1 with hydrochloric acid. The precipitate (10.5 g, anthrone value 0.6%) was removed by filtration. The filtrate was extracted with chloroform (two 500-ml portions), neutralized with lithium hydroxide solution, and evaporated *in vacuo* below 35°. The resultant syrup was thoroughly dried *in vacuo* over phosphorus pentoxide and extracted with three 500-ml portions of 1:1 acetone-ethanol. The residue (12.3 g) contained phosphorylated and nonphosphorylated oligosaccharides contaminated with some inorganic material. Found: N, 1.01; P, 1.50; anthrone, 30.0; ash, 17%.

Separation of Phosphorylated from Nonphosphorylated Oligosaccharide.—A column (3.6 × 80 cm) of Dowex-2 (HCO_3^-) resin was prepared. Additional resin which had been used to neutralize the eluate from the Amberlite IRC-50 column was placed on top and a concentrated solution containing the oligosaccharide mixture (10.0 g) was applied to the column. The column was eluted with water. Fractions were collected until the eluate contained less than 0.05 mg of oligosaccharide per ml. All fractions were combined and lyophilized to provide the nonphosphorylated oligosaccharide (5.1 g). Found: N, 1.16; P, 0.01; anthrone, 22.0; ash, 4.0%.

The column was then eluted with 5% aqueous ammonium acetate solution. Fractions were again collected and evaporated. From the solid thus obtained, the excess ammonium acetate was sublimed *in vacuo* at 40°. The elution was continued until negligible quantities of oligosaccharide remained after sublimation. The combined residues consisted of phosphorylated oligosaccharide contaminated with traces of ammonium acetate. Use of a 10% solution of ammonium acetate for elution caused disruption of the column of resin, while replacement of the ammonium acetate by 5%

TABLE I
FRACTIONATION OF 0.8-G SAMPLE OF OLIGOSACCHARIDE ON CARBON-CELITE

Ethanol (%)	Volume (ml)	Weight of Fraction (mg)	Recovery of Sample (%)	Major Component
0	500	31.8	4.0	O[a]
5	500	97.6	12.2	A
10	500	242.0	30.2	B
15	500	83.2	10.4	C
20	500	99.1	12.4	D
30	500	52.1	6.5	E
40	500	25.0	3.1	F

[a] Inorganic contaminants plus monosaccharide.

TABLE II
CARBOHYDRATE COMPOSITION OF FRACTIONS FROM CARBON-CELITE COLUMN

Fraction	Relative Proportions of Monosaccharides			
	Galactose	Mannose	Arabinose	Fucose
A	0.5	4	0	0
C, D	10	2	3	1
E	10	2	4	1

formic acid resulted in extensive decomposition of the phosphorylated oligosaccharide.

Fractionation of Oligosaccharide

On Carbon-Celite.—A column (3.6 × 15 cm) was filled with a 2:1 mixture of Darco G-60 and Celite 535 as described by Whistler and Durso (1950). An 0.8-g sample of oligosaccharide (obtained by barium hydroxide hydrolysis) was dissolved in a small volume of water. The solution was applied to the column, which was eluted first with water and then with increasing concentrations of aqueous ethanol. A maximum flow rate of 50 ml/hour was attained initially but this decreased rapidly as the ethanol concentration increased, 100-ml fractions of eluate were collected. The material recovered was distributed in seven peaks. A negligible amount of material was eluted after the ethanol concentration exceeded 40%. The results of the fractionation are summarized in Table I. Samples of the various fractions were hydrolyzed with 0.5 N hydrochloric acid in a sealed tube on a steam bath for 12 hours. The neutralized hydrolysates were chromatographed on paper by method 2. Relative proportions of individual sugars were estimated by visual comparisons with standard chromatograms. The results are summarized in Table II.

On Dowex-2 (HCO_3^-).—A column (3.6 × 75 cm) was packed with Dowex-2 resin (200–400 mesh) which had been well washed to remove fines and converted to the bicarbonate form. The column was used immediately. A sample of the nonphosphorylated oligosaccharide mixture (4.1 g) was suspended in 5 ml of water, the insoluble material (190 mg) was filtered off, and the filtrate was placed on the resin. The column was eluted continuously with water (16 liters) at a rate of 200 ml/hour until all the sample was recovered. Fractions (250 ml) each were collected, concentrated, and lyophilized. Six peaks were eluted (see Table III and Fig. 1). Samples (100 mg) of each of the peak fractions were hydrolyzed and the neutralized hydrolysates were subjected to preparative chromatography on Whatman No. 3 paper by method 2. Individual monosaccharides and unhydrolyzed glucosaminido-glucuronido-inositol were eluted and weighed. (In each case control chromatograms were run.) The crude analytical data thus obtained are recorded in Table IV. The chromatographic behavior of the intact fractions is summarized in Table V.

CHARACTERIZATION OF TETRASACCHARIDE FRACTION (COMPONENT B): PURIFICATION.—Fraction B (1.0 g) was dissolved in water (1 ml), applied to a column (4.8 × 105 cm) of G-25 Sephadex (medium grade) and eluted with water. After a volume equal to the hold-up volume of the column had been passed, 25-ml fractions were collected. All the material was eluted in a single peak with only a slight inflection at the leading edge. Samples of the individual fractions were hydrolyzed and chromatographed by method 2. The 1100- to 1200-ml eluate fraction (12% of sample) containing material from the leading edge of the peak produced a strong spot due to mannose, a faint spot corresponding to galactose, and a barely detectable spot due to arabinose. The 1200- to 1300-ml eluate fraction (72% of sample) produced only one very intense spot due to mannose. Lyophilization of this fraction gave the purified tetrasaccharide (component B) as a pure white powder, mp 236–239° (decomp), $[\alpha]_D^{25}$ = +107° (0.13% solution in water). Attempts to crystallize this material were unsuccessful.

TABLE III
FRACTIONATION OF 4.1-G SAMPLE OF OLIGOSACCHARIDE ON DOWEX-2 (HCO_3^-)

Fraction Number	Major[a] Component	Weight (g)	Per Cent of Sample	Glucosamine (%)	Glucuronic Acid (%)
1–3	F[b]	0.428	12	8	
4–8	E	0.536	14	15	
9–14	D	0.449	10	16	18
15–21	C	0.388	10	18	
22–40	B	1.650	41	25	26
41–64	A	0.351	9	32	30
Insoluble material		0.164	4		

[a] As determined by paper chromatography and analytical data. [b] This fraction contained some inorganic material.

Analyses: Found (after lyophilization): C, 39.02; H, 6.48; N, 1.86%. Found (after drying at 78°, 0.05 mm for 18 hours): C, 41.77; H, 6.36; N, 2.04%. Calcd. for $C_{24}H_{41}NO_{21}.3\ H_2O$: C, 39.29; H, 6.45; N, 1.91. Calcd. for $C_{24}H_{41}NO_{21}.{}^1/_2\ H_2O$: C, 41.90; H, 6.14; N, 2.04.

N-ACETYL TETRASACCHARIDE.—Acetic anhydride (1.6 ml) was added dropwise to a stirred cold (0°) solution of crude tetrasaccharide (1.84 g) and sodium bicarbonate (3.44 g) in water (25 ml) over a period of 5 minutes. The solution was stirred for an additional hour and then left at room temperature overnight. Acetic acid was added to destroy excess bicarbonate ion and sodium ion was removed with a column of IR-120 H^+. Concentration of the eluate to 10–15 ml

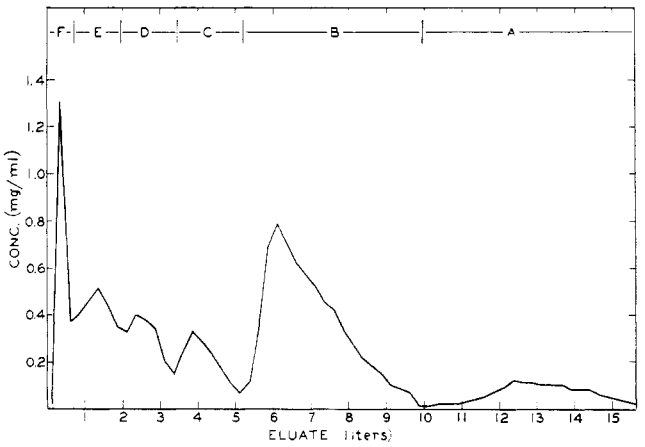

FIG. 1.—Fractionation of oligosaccharide on Dowex-2 (HCO_3^-). Experimental conditions are given in text.

TABLE IV
CARBOHYDRATE COMPOSITION OF INDIVIDUAL OLIGOSACCHARIDE FRACTIONS

Component	Galactose (%)	Mannose (%)	Arabinose (%)	Fucose (%)	GGI[a] (%)
B	2	22	0	0	57
C	24	4	5	4	36
D	47	5	5	5	30
E	44	2	12	2	26
F	42				

[a] Glucosaminido-glucuronido-inositol.

TABLE V
BEHAVIOR OF OLIGOSACCHARIDE FRACTIONS ON PAPER CHROMATOGRAPHY WITH 1-BUTANOL-PYRIDINE-WATER (6:4:5)

Fraction	R_F	Major Component
A	0.24	Trisaccharide[a]
B	0.18	Tetrasaccharide[b]
C	0.14	Pentasaccharide
D	0.10	Hexasaccharide
E	0.06	Heptasaccharide
F	0.02	Octasaccharide

[a] Glucosaminido-glucuronido-inositol. [b] (Mannosido)-glucosaminido-glucuronido-inositol.

followed by addition of ethanol to turbidity produced crystals immediately. These were filtered and washed with ethanol-water (6:4) and dried. The product weighed 1.61 g. A small sample was recrystallized from aqueous ethanol as previously. The purified material had mp = 191–194° d; $[\alpha]_D^{20}$ = +143° (0.7% solution in water) and gave strong infrared bands for hydroxyl, carboxyl, and amide.

Anal. Calcd. for $C_{26}H_{43}O_{22}N$ (721.6): C, 43.25; H, 6.01; N, 1.94. Found: C, 42.93; H, 6.41; N, 1.87.

N-ACETYL CARBOXYL–REDUCED TETRASACCHARIDE.—*N*-Acetyl tetrasaccharide was converted to the methyl ester with diazomethane by the method of Roper and Ma (1957). A suspension of 0.55 g of *N*-acetyl derivative in 50 ml of methanol was stirred (magnetic stirrer) while diazomethane (from a boiling ethereal solution) was introduced through a gas inlet tube below the surface of the methanol. When the color of diazomethane persisted for several minutes, the inlet tube was removed and replaced by a stopper. After several hours a few drops of acetic acid were added and the reaction mixture was concentrated to a small volume. The resulting solids were taken up in 20 ml of water, the solution was cooled to 0°, and a solution of 0.5 g of sodium borohydride in 5 ml of water was slowly added. After 15–18 hours, excess borohydride ion was destroyed with acetic acid and sodium ion was removed with IR-120 H^+. The effluent was concentrated to a small volume, passed through a *thin* pad of carbon, and concentrated to dryness. The residue was distilled with several 50-ml portions of methanol and the treatment with diazomethane and sodium borohydride was repeated. The final product was taken up in a small volume of water and ethanol was added to turbidity. Crystallization occurred within 1 hour giving 0.42 g of product. Once-recrystallized material showed mp 191–193° d, $[\alpha]_D^{20}$ = +136° (1% solution in water). Bands for hydroxyl and amide were present in the infrared spectrum. Both ester and carboxyl bands were completely absent. Acid hydrolysis produced equal amounts (visual estimation) of glucosamine, glucose, inositol, and mannose as determined by paper chromatography with authentic standards: pyridine–ethyl acetate–water–acetic acid (5:5:3:1).

Anal. Calcd. for $C_{26}H_{45}O_{21}N$ (707.7): C, 44.12; H, 6.41; N, 1.98. Calcd for $C_{26}H_{45}O_{21}N.{}^1/_2\ H_2O$ (716.7): C, 43.57; H, 6.47; N, 1.95. Found: C, 43.69; H, 6.46; N, 2.05.

On Sephadex G-25.—A column (4.8 × 120 cm) was packed with a 100-cm length of Sephadex G-25 (medium grade). (V_t calculated, 1800 ml; V_0 estimated, 675 ml.) The elution pattern of the column was checked with glucosamine and raffinose which appeared, respectively, in the 1350- to 1450-ml and 1200- to 1300-ml fractions of eluate. Tetrasaccharide partially purified over Dowex-2 (HCO_3^-) (see previous section) gave the major peak in the 1200- to 1300-ml fraction. A small amount of pentasaccharide contaminant was separated in this way, appearing in the 1100- to 1200-ml fraction. Following these control experiments the column was used to fractionate oligosaccharide and phosphorylated oligosaccharide samples.

In a typical experiment a solution of 1.0 g of oligosaccharide (or phosphorylated oligosaccharide) in 2.0 ml of water was applied to the column which then was eluted with water. After the hold-up volume was collected (in one or two fractions), the remaining eluate was collected in 50-ml fractions. Each of the frac-

TABLE VI
FRACTIONATION OF 1.0-G SAMPLES OF OLIGOSACCHARIDE AND PHOSHORYLATED OLIGOSACCHARIDE ON SEPHADEX G-25.

Fraction Number	Volume (ml)	Oligosaccharide Weight (mg)	Oligosaccharide N (%)	Phosphorylated Oligosaccharide (wt in mg)
1	600	30.		5.6
2	150	19.4		31.7
3	100	15.6		56.1
4	50	13.7	0.98	37.9
5	50	19.5		38.5
6	50	50.9	1.10	35.4
7	50	96.5	1.16	41.0
8	50	98.7	1.28	47.7
9	50	171.3		68.7
10	50	197.5	1.85	99.8
11	50	94.2		144.4
12	50	43.3	2.45	140.9
13	50	19.2		83.0
14	50	20.0		36.9
15	50	11.7		58.8
16	50	4.0		48.7
	Total recovery	905.5		975.1

TABLE VII
CHROMATOGRAPHIC BEHAVIOR OF OLIGOSACCHARIDE FRACTIONS FROM SEPHADEX G-25 COLUMN

Fraction Number	R_F	Major Component(s)
3	0.02	Octa and higher saccharides
5	0.05	Heptasaccharide
8	0.06, 0.10	Hepta- and hexasaccharide
10	0.10, 0.14, 0.18	Hexa-, penta-, and tetrasaccharide
11	0.14, 0.18	Penta- and tetrasaccharide
13	0.24	Trisaccharide

TABLE VIII
CARBOHYDRATE COMPOSITION OF OLIGOSACCHARIDE FRACTIONS FROM SEPHADEX G-25

Fraction Number	Relative Proportions of Monosaccharides			
	Galactose	Mannose	Arabinose	Fucose
3	10	1	8	1
6	10	1	5	1
7	10	1	4	1
8	10	1	3	1
9	7	4	1	0
11	1	5	0	0
13	0.5	3	0	0

tions was concentrated *in vacuo* and finally dried by lyophilization.

The column data are summarized in Table VI. Data on the oligosaccharide fractions are given in Tables VII and VIII, and on the phosphorylated oligosaccharide fraction in Table IX.

Although clean separation of individual oligosaccharides was not achieved, it is obvious that the first fractions contained the higher oligosaccharides with progressive decrease in molecular weight of later fractions and with the main tetrasaccharide fraction appearing in the 1150- to 1250-ml fractions.

TABLE IX
ANALYSES OF FRACTIONS FROM CHROMATOGRAPHY OF PHOSPHORYLATED OLIGOSACCHARIDE ON SEPHADEX G-25

Fraction Number	N (%)	P (%)	Glucuronic Acid (%)	N-P-Glucuronate (molar ratio)
5	1.08	2.46	16	1:1.03:1.07
7	1.12	2.80	16	1:1.13:1.02
9	1.19	2.95	18	1:1.12:1.10
11	1.46	3.02	22	1:1.01:1.09
13	1.60	3.43	23	1:0.97:1.04

The phosphorylated oligosaccharide gave a pattern of distribution very similar to that of the nonphosphorylated material and the molar ratios of glucosamine, glucuronic acid, and phosphorus support the conclusion that the phosphorylated material has the same basic carbohydrate structure as the nonphosphorylated oligosaccharide.

REFERENCES

Bitter, T., and Ewins, R. (1961), *Biochem. J. 81*, 43P.

Carter, H. E., Brooks, S., Gigg, R. H., Strobach, D. R., and Suami, T. (1964), *J. Biol. Chem. 239*, 743.

Carter, H. E., Celmer, W. D., Galanos, D. S., Gigg, R. H., Lands, W. E. M., Law, J. H., Mueller, K. L., Nakayama, T., Tomizawa, H. H., and Weber, E. (1958a), *J. Am. Oil Chemists' Soc. 35*, 335.

Carter, H. E., Galanos, D. S., Hendrickson, H. S., Jann, B., Nakayama, T., Nakazawa, Y., and Nichols, B. (1962), *J. Am. Oil Chemists' Soc. 39*, 107.

Carter, H. E., Gigg, R. H., Law, J. H., Nakayama, T., and Weber, E. (1958b), *J. Biol. Chem. 233*, 1309.

Harris, W. D., and Popat, P. (1954), *J. Am. Oil Chemists' Soc. 31*, 124.

Lauter, C. J., and Trams, E. G. (1962), *J. Lipid Res. 3*, 136.

McKibben, J. M., and Taylor, W. E. (1949), *J. Biol. Chem. 178*, 291.

Roper, R., and Ma, T. S. (1957), *Microchem. J. 1*, 245.

Whistler, R. L., and Durso, D. J. (1950), *J. Am. Chem. Soc. 72*, 677.