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Note

Identification of iduronic acid as a constituent of the "type-specific" polysaccharide of *Clostridium perfringens* Hobbs 10*

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L-Iduronic acid has been encountered infrequently in Nature It was first recognized by Hoffman *et al* [1] as a major constituent of dermatan sulfate, a connective tissue polysaccharide, because of its low color-yield in the carbazole reaction Recently, L-iduronic acid has been identified as a constituent of heparin and heparan sulfate[2]. Two of these were the only documented cases for the biological occurrence of L-iduronic acid until A S Jones *et al* [3] reported the apparent isolation of dermatan sulfate from *Clostridium perfringens* NCTC 10578 Their data were based on the analysis of an acidic polysaccharide which represented an extremely low percentage of the bacterial cell-mass The small amount of material isolated precluded extensive fractionation or the determination of the degree of homogeneity The possible relationship of the dermatan sulfate isolated to the beef extract in the growth medium was not even considered These points are of importance, because of the possibility of aggregation of extracellular dermatan sulfate with polysaccharides of the cell surface[4]

An investigation of the "type-specific" polysaccharide of *C perfringens* Hobbs 10, purified chromatographically and found immunologically homogeneous, revealed the presence of a uronic acid constituent that exhibited a low carbazole value This observation was reminiscent of the results of Jones *et al* [3], except that the uronic acid was obtained from an unequivocal source, a weakly acidic, "type-specific" polysaccharide of high purity[5] The purpose of this Note is to present evidence for the occurrence of iduronic acid as a constituent of the "type-specific" polysaccharide of *Clostridium perfringens* Hobbs 10

EXPERIMENTAL

General — Purified "type-specific" polysaccharide, obtained from *C perfringens* Hobbs 10, was utilized in this study[5]. Paper chromatography was performed

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by the descending technique on Whatman No 1 filter paper, with the following solvent systems *A*, 6 4 3 (v/v) 1-butanol–pyridine–water, *B*, 5 5 3 1 (v/v) ethyl acetate–pyridine–water–acetic acid, and *C*, 10 3 1 (v/v) 1-butanol–acetic acid–water Sugars were detected with alkaline silver nitrate Uronic acid was estimated by the carbazole[6] and orcinol[7] reactions, with D-glucuronic acid as the standard Isolation of the free uronic acid constituent was accomplished by the method of Jeffrey and Rientis[8] To ascertain the efficiency of the isolation procedure, a sample of D-glucuronic acid was treated in a similar way

Crystalline 1,2-*O*-isopropylidene-*β*-L-idofuranose, 1,2-*O*-isopropylidene-*β*-L-idofuranurono-6,3-lactone, and an amorphous sample of dermatan sulfate were gifts from Dr E A Davidson, M S Hershey Medical Center, Hershey, Pennsylvania Free L-idose and L-iduronic acid were obtained by hydrolyzing their corresponding isopropylidene derivatives (9 mg) with Dowex 50 (H^+) resin (100 mg) plus 0 01M hydrochloric acid (1 ml) for 30 min and 24 h, respectively, at 100° L-Idose (10 mg) was almost quantitatively converted into 1,6-anhydro-L-idopyranose by treatment with Dowex 50 (H^+) resin (100 mg) plus 0 05M hydrochloric acid (1 ml) for 24 h at 100° Reduction of carboxyl groups was accomplished on 50 mg of polysaccharide with sodium borotritiide (New England Nuclear Corporation) according to the procedure of Sutherland[9] The reduced polysaccharide was hydrolyzed with Dowex 50 (H^+) resin (100 mg) plus 0 05M hydrochloric acid (1 0 ml) in a sealed tube for 24 h at 100° Hydrochloric acid was removed by repeated addition and evaporation of absolute alcohol *in vacuo* A sample of dermatan sulfate was treated similarly, as a control

Radioactive samples were applied to Whatman No 1 filter paper, with idose or 1,6-anhydro-idopyranose as the internal standard, and the paper was developed with solvent *A* The paper was dried, and each sample lane was divided in halves One half of each lane was cut into 1-cm sections, these were placed in 15 ml of Aquasol Universal L S C Coctail (New England Nuclear Corporation), and the radioactivity was determined in a liquid scintillation-counter (Packard, Tri-Carb Model 2420) The other half of each lane was stained with alkaline silver nitrate The positions of the radioactive peaks were compared with the location of the stained, internal standards

Gas–liquid chromatography (g l c) was performed with a Beckman Model GC-65 instrument equipped with dual, flame-ionization detectors Analyses were conducted with 6-ft columns of 3% of SE-52 on Chromosorb W (100–120 mesh, high performance), with helium as the carrier gas The per(trimethylsilyl) ethers of the samples were introduced at 140°, and this temperature was held for 7 min The column temperature was programmed to rise to a maximum of 180° at a rate of 2 75° min^{-1} after the hold interval[10]

RESULTS AND DISCUSSION

The uronic acid isolated gave a "carbazole to orcinol" ratio of 0 32 1 L-Iduronic acid determined in the same way gave a "carbazole to orcinol" ratio of 0 31 1

The quantity of uronic acid isolated corresponded to 5 6% of the dry weight of the purified polysaccharide This value can be restated as 7%, if a correction factor based on 80% recovery of glucuronic acid is applied

Paper chromatography of the isolated uronic acid in solvents *B* and *C* demonstrated that its mobility coincided with that of L-iduronic acid (see Table I)

TABLE I

PAPER-CHROMATOGRAPHIC IDENTIFICATION OF IDURONIC ACID[a]

Uronic acid	*Solvent* B	*Solvent* C
Galacturonic	0 90	0 91
Glucuronic	**1 00**	**1 00**
Guluronic	1 03	1 02
Mannuronic	1 16	1 02
Iduronic	1 26	1 11
Unknown	1 27	1 11

[a]The values given are the mobilities relative to that of D-glucuronic acid as unity The lactones have run off the paper

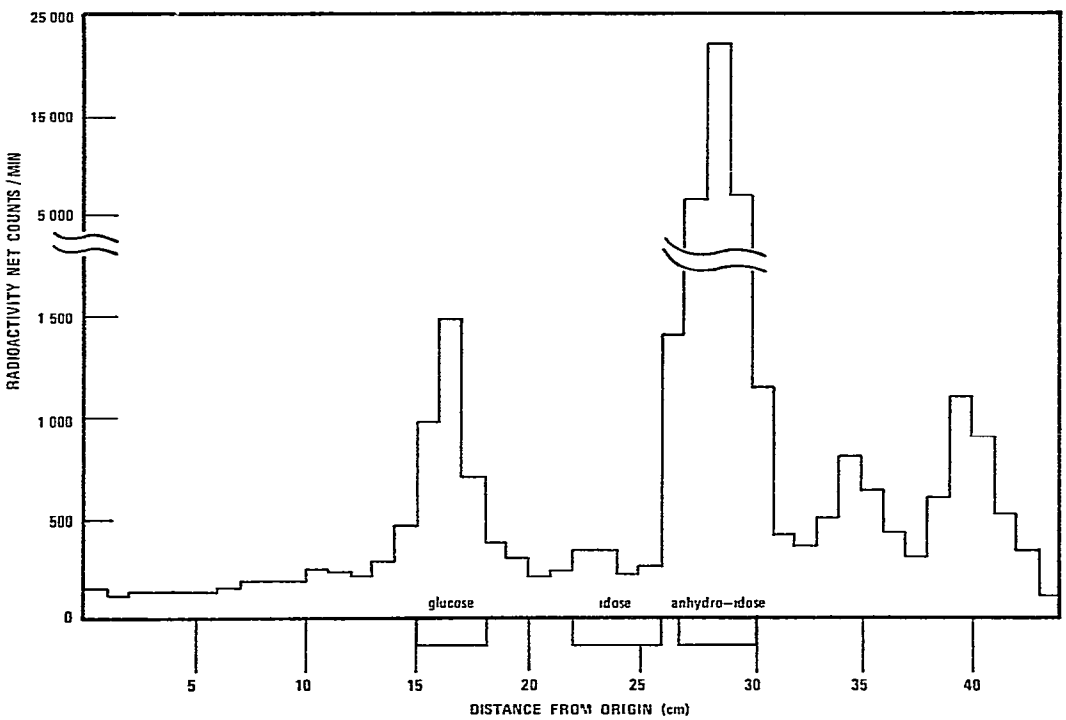

Fig 1 Paper-chromatographic identification of tritiated 1,6-anhydro-idopyranose in solvent *A*

Paper chromatography of the monosaccharides derived from the hydrolysis of the sodium borotritiide-reduced polysaccharide showed a single, major component whose mobility coincided with that of 1,6-anhydro-L-idopyranose (see Fig 1) A small quantity of tritiated glucose was detected also Similar results were obtained with dermatan sulfate A second aliquot of the reduced polysaccharide, analyzed by g l c, contained a monosaccharide not present in hydrolyzates of the untreated samples The relative retention-time of the new constituent coincided with that of 1,6-anhydro-idopyranose (see Table II)

TABLE II

GAS–LIQUID CHROMATOGRAPHY[a]

Monosaccharide	*Standard*	*Sample*	
		Reduced	*Untreated*
1,6-Anhydro-idopyranose	0 61	0 61	absent
α-Mannose	0 81	absent	absent
α-Galactose	0 94	0 95	0 94
α-Glucose	**1 00**	**1 00**	**1 00**
β-Galactose	1 03	1 03	1 03
β-Glucose	1 22	1 22	1 22

[a]The values given are retention times relative to that of α-D-glucose as unity

The three separate lines of evidence presented in this Note establish that an iduronic acid is a constituent (7%) of the "type-specific" capsular polysaccharide of *Clostridium perfringens* Hobbs 10 No attempt has yet been made to determine the configuration of the iduronic acid moiety

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