mixtnre was protected against light and frequently shaken. Aliquots of 5 ml were titrated by 0.01 N sodium hydroxide after reduction of the periodate by ethylene glycol; 48 h 5.22 ml; 92 h 5.74 ml (170 h, yellow-brownish colour of solution, side reactions). The amount of formic acid liberated (92 h) corresponds to 41.2 % terminal hexose units. (b) *Periodate uptake.* The galactomannan (80 mg) was dissolved in 45 ml of water, sodium meta-periodate (200 ml, 0.01 M) was added and water to make 250 ml. Aliquots of 25 ml were titrated by the arsenite method.[6] Results from a typical experiment given as moles of periodate reduced per anhydrohexose unit 0.67 (1 h); 1.03 (4 h) 1.06 (24 h) 1.11 (48 h); 1.14 (70 h).

Methylation and analysis of the methylated sugars. The polysaccharide (1.25 g) gave by methylation [2] a product (0.7 g) with the following properties; white, crisp powder, $[\alpha]_D^{24}$ + 74.4° (c 1.20 chloroform); MeO 43.55 % After complete hydrolysis the mixture of methyl derivates on paper chromatograms (benzene--ethanol-water, 167: 47: 15 v/v) behaved exactly as a mixture of the following reference substances; (I) 2,3-dimethyl-D-mannose (R_g 0.09), (II) 2,3,6-trimethyl-D-mannose (R_g 0.41), (III) 2,3,4,6-tetramethyl-D-galactose (R_g 0.88) (R_g = 1.00 for tetramethyl-D-glucopyranose). Their molecular proportion was determined to be (I) 3.00: (II) 1.00: (III) 3.38, respectively, as mean values of replicate analysis. The quantitative method used was oxidation with hypoiodite [7] as adapted to methylated mannose derivatives.[2] Fraction (III) was further characterized as the crystalline derivative N-phenyl--2,3,4,6-tetramethyl-D-galactopyranosylamine; m.p. 204°, $[\alpha]_D^{20}$ −142° (c 0.50, pyridine);[8] MeO calc. 39.81 %, found 37.8 %.

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Uridine Diphosphate Glucose in Cellulose Forming Cultures of *Acetobacter xylinum*

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Glaser [1] has shown that a particulate enzyme preparation from *Acetobacter xylinum* catalyses the synthesis of cellulose from uridine diphosphate glucose, UDPG. It is therefore of interest to show whether UDPG actually is present in cellulose forming cultures of *A. xylinum*. Colvin *et al.*[2,3] examined the extracellular medium of active glucose metabolising cells of *A. xylinum* and found no UDPG present. Weigl [4] examined the cellulose pellicles from cultures of *A. xylinum* on a *p*-aminobenzoic acid dependent medium and was unable to detect UDPG. This paper reports the presence of UDPG in cultures of *A. xylinum* with evident cellulose formation. The UDPG is found in a perchloric acid extract of the cellulose pellicles and also, in lower concentration, in the liquid phase of the cultures.

Culture conditions. The bacterium, *Acetobacter xylinum* ATCC 10245, was grown on a synthetic medium [4] containing 4 % glucose, 1 % ethanol, inorganic salts, and small amounts of *p*-aminobenzoic acid. The liquid phase of a three-day culture with evident cellulose formation served as inoculum. Each flask containing 100 ml medium was inoculated with 10 ml inoculum. The cultures were incubated quiescently at 29°C.

Isolation of UDPG. Four days after inoculation the pellicles from 5 cultures were withdrawn. The pellicles with included bacteria and substrate were treated in a Waring Blendor with 0.5 l distilled water. Perchloric acid was added at 0°C to give a concentration of 6 % w/w and the mixture was stirred for 30 min. After centrifugation the supernatant liquid was neutralized with KOH to pH 6.0. After removal of precipitated $KClO_4$ the solution was reduced in volume to 100 ml and precipitated $KClO_4$ again removed. Nucleotides were adsorbed on 0.5 g of norite and the norite washed with 100 ml of distilled water. UDPG and some pigments were then desorbed with 50 ml portions of 50 % ethanol. Each ethanol

extract was reduced to a small volume and applied as a single spot to a Whatman No. 1 paper for chromatography. Descending chromatography was used and solvent (II) (see below) was employed. A contact print of the chromatogram in monochromatic light of 254 mμ revealed only one spot in the nucleotide area. The position of this spot was that of authentic UDPG.

Identification of UDPG. The spot of the isolated compound was cut out from the chromatogram and eluted. Rechromatography in three different solvents: (I) 96 % ethanol – 1 M ammonium acetate, pH 7.5 (5:2, by vol.); (II) 96 % ethanol – 1 M ammonium acetate buffer, pH 3.8 (5:2, by vol.)[5]; (III) isopropanol -saturated ammonium sulphate-water (2:79:19, by vol)[6] gave identical R_F-values with authentic UDPG, as shown in Table 1. The ultraviolet

Table 1. Chromatographic mobilities (R_F-values) of the isolated compound and authentic UDPG.

	solvent (I)	solvent (II)	solvent (III)
Isolated compound	0.23	0.26	0.77
Authentic UDPG	0.23	0.25	0.77

absorption spectra at pH 2 and pH 12 in the range 220 – 300 mμ were found identical with those of authentic UDPG. The UDPG isolated from several cultures was pooled to obtain 1.0 μmole and subjected to analysis after rechromatography in solvents (I) and (II). *Uridine* was determined on the basis of ultraviolet absorption taking the molar absorbancy index of uridine phosphates at pH 2 and 260 mμ as $a_M = 10.0 \times 10^3$ l mole^{-1} cm^{-1}. *Phosphate* was determined by the method of Allen.[7] (Acid labile phosphate after hydrolysis for 15 min in 1 N perchloric acid at 100°C and total phosphate after hydrolysis for 1 h in 70 % w/w of perchloric acid at 100°C in a sealed tube). *Glucose* was released by hydrolysis for 10 min in 0.01 N H_2SO_4 at 100°C. The hydrolysate was neutralized with $Ba(OH)_2$, the precipitated $BaSO_4$ removed, and glucose identified by paper chromatography in butanol-pyridine-water (6:4:3, by vol.)[8] using $AgNO_3$[9] as location reagent. The amount of glucose released was determined by the method of Park and Johnson.[10]

The ratio uridine:acid labile phosphate: total phosphate:glucose for the isolated compound was 1.0:0.9:1.9:1.1; while authentic UDPG gave 1.0:0.9:2.0:0.9.

The amount of UDPG present in 5 pellicles varied from 0.04 to 0.16 μ moles.

UDPG could also be detected in the liquid phase of 4 days cultures but the concentration was about one tenth of that found in the pellicles.

Elbein, Barber and Hassid[11] have recently shown that a synthesis of cellulose can be accomplished from guanosine diphosphate glucose, GDPG, with a particulate enzyme preparation from beans. As other nucleotide sugars are shown to be ineffective this indicates that GDPG is the glucosyl donor in cellulose formation in higher plants. By the procedure employed in the present paper there was no indication of the presence of GDPG in cellulose forming cultures of *Acetobacter xylinum*.

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