Thymidine derivatives of this type are uncommon and only recently have thymidine diphosphate rhamnose[1] and related deoxysugar nucleotides[17] been described. Guanosine diphosphate mannose[18] is the only other nucleotide containing mannose. Thymidine diphosphate mannose is of interest as a possible intermediate in the biosynthesis of streptomycin B (α-D-mannopyranosyl-streptomycin)[19], which occurs together with streptomycin in this micro-organism.

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The isolation of guanosine diphosphate colitose from *Escherichia coli*

The specific immunochemical reactions exhibited by bacterial endotoxins (lipopolysaccharides) are primarily determined by the 3,6-dideoxyhexose components of these substances[1]. LUDERITZ *et al.*[2] reported that colitose (3,6-dideoxy-L-galactose) is the dideoxyhexose of a lipopolysaccharide isolated from *Escherichia coli* O111-B_4. A colitose-containing nucleotide, guanosine diphosphate colitose, has now been isolated from this organism*.

Cells were grown in a continuous-culture apparatus, harvested during exponential growth and washed with water. 1 kg (wet weight) of cells was extracted with cold 10% trichloroacetic acid, the precipitate removed by centrifugation and the supernatant fluid extracted 5 times with ice-cold ether. The ultraviolet-absorbing material in the neutral extract was fractionated on Dowex-1 (Cl^- form) resin using LiCl as

* A culture of *E coli* O111-B_4 was kindly supplied by Dr Pearl L Kendrick, School of Public Health, University of Michigan, Ann Arbor, Mich

the eluting agent. The crude nucleotide fraction, eluted with 0.25 *M* to 0.35 *M* LiCl, was concentrated and the lithium salts of the nucleotides were precipitated. Analysis of a portion of this fraction, after hydrolysis at 100° in 0.01 *N* HCl for 10 min, indicated the presence of colitose as determined by paper chromatography and the reaction obtained with 2-thiobarbituric acid*. No other reducing substances were detected on the chromatograms with the ammoniacal $AgNO_3$ reagent[4]. Colitose was not detected in the crude nucleotide fraction prior to acid hydrolysis Further fractionation of the crude lithium salts by treatment with charcoal, paper chromatography and paper ionophoresis yielded 2.5 μmoles of a colitose-containing nucleotide Paper chromatography in two solvent systems and ionophoresis** indicated that the substance was homogeneous; in each case, hydrolysis of the ultraviolet-absorbing spot yielded colitose The ultraviolet-absorption spectrum of the nucleotide was identical with that of guanosine monophosphate at pH's 1.0, 7.0 and 11.0. Analysis of the material indicated the following molar ratios. guanine, 1.0; organic phosphorus, 1 97; colitose, 0.95. The colitose moiety was further characterized, following hydrolysis of the nucleotide, by comparison with authentic colitose*** in three paper-chromatographic solvent systems. Similarly, the only ultraviolet-absorbing material present in such an hydrolysate was chromatographically indistinguishable from guanosine diphosphate[5]. The nucleotide exhibited a negative reaction with diphenylamine although deoxyguanosine monophosphate gave a typical blue color under the same conditions. The nucleotide apparently is guanosine diphosphate colitose.

The occurrence of a guanosine nucleotide containing colitose suggests a possible metabolic relationship to the other known guanosine sugar nucleotides such as guanosine diphosphate mannose and guanosine diphosphate fucose, the relationship between the latter compounds already has been demonstrated[6]

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* Oxidation of colitose with periodic acid yields malonyl dialdehyde which reacts with 2-thiobarbituric acid to give a pink-colored complex absorbing maximally at 532 mμ (ref. 3)

** Chromatography of sugars was carried out on Whatman No. 1 paper in the following solvent systems: (*a*) butanol (6)–pyridine (4)–water (3), (*b*) ethyl acetate (3)–acetic acid (1)–water (3), (*c*) butanol (10)–ethanol (1)–water (2). Paper ionophoresis was carried out on Whatman No. 3MM paper at 10 V/cm in 0.05 *M* phosphate buffer, pH 7.4

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