

(g) In doubtful cases, it would be convenient to determine whether the saccharose is utilized by the  $\alpha$ -glucosidase or by  $\beta$ -fructosidase: I consider the qualitative analysis of the culture medium by means of paper chromatography, when the phase of positive development has been reached, as sufficient proof.

(h) It is necessary not to confuse the adaptive fermentation from saccharose by  $\beta$ -fructosidase (*S. fragilis*) with the saccharose utilization by  $\alpha$ -glucosidase (*S. italicus*, *S. oleagenosus* and *S. hienipiensis*), though this reaction is also inducible.

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### Adenosine Diphosphate Glucose and Glucoside Biosynthesis

URIDINE diphosphate glucose (UDPG) has been found to act as glucose donor in a number of enzymatic reactions, such as glucoside formation in plants<sup>1</sup> and in locust<sup>2</sup>, sucrose and starch synthesis in plants<sup>3,4</sup> and glycogen formation in mammals<sup>5</sup>.

Further work showed that adenosine diphosphate glucose (ADPG) is a better substrate for starch synthesis than UDPG, but half as effective as the latter for glycogen formation and nearly ineffective for sucrose synthesis<sup>6</sup>.

Arbutin or very similar glucosides were reported to occur naturally in seeds and locusts<sup>7,8</sup>. Since arbutin is formed with enzymes either from wheat germ or locust it was considered of interest to test ADPG with these systems, which are known to use UDPG as substrate.

With the enzyme from locust<sup>2</sup> ADPG proved to be ineffective for glucoside synthesis. On the other hand, using the enzyme from wheat germ<sup>1</sup>, ADPG led to the formation of two glucosides which were indistinguishable from those formed when UDPG was used as substrate, that is, arbutin and the corresponding gentiobioside. All experiments were carried out with enzyme extracts and methods described previously<sup>1,2,6</sup>. For the characterization of the glucosides the reaction with bromine was also used<sup>7,8</sup>.

The quantitative results obtained are shown in Tables 1 and 2. It may be observed that the synthesis of glucosides and gentiobiosides is faster when ADPG is substituted for UDPG, but the difference in rates of transfer is not so great as in starch synthesis<sup>6</sup>.

Other nucleotides were also assayed as glucose donors in glucoside synthesis. Deoxy-ADPG, UDP-maltose and an isomer of UDPG in which the glucose is joined to UDP through position 6 gave negative results. Guanosine diphosphate glucose, and to a lesser extent cytidine diphosphate glucose, act slightly as glucose donors, as estimated by nucleoside diphosphate formation in the presence of hydroquinone.

The reactions of ADPG and other nucleotides with different phenols and enzymes from several sources are now being examined.

Table 1. FORMATION OF GLUCOSIDES AND GENTIOBIOSIDES

Substrates		$\mu$ moles formed	
Phenol	Nucleotide	Glucoside	Gentiobioside
Hydroquinone	UDPG	0.12	0.03
Hydroquinone	ADPG	0.15	0.05
Arbutin	UDPG	—	0.09
Arbutin	ADPG	—	0.15

The system contained: 0.5  $\mu$ mole, ADPG or UDPG; 0.8  $\mu$ mole, hydroquinone or arbutin; 0.15  $\mu$ mole, ethylenediamine tetraacetic acid; 0.15  $\mu$ mole, cysteine; 30  $\mu$ moles *tris*(hydroxymethyl)aminomethane-maleate buffer (pH 6.8); 0.05 ml., wheat germ enzyme (fraction I), (ref. 1). Total volume, 0.2 ml. The reaction mixture was incubated 60 min. at 37° and chromatographed. The spots containing glucoside and gentiobioside were eluted and estimated as previously reported (ref. 1).

Table 2. FORMATION OF UDP OR ADP

Substrates		$\mu$ moles of UDP or ADP formed		
Phenol	Nucleotide	10 min.	25 min.	40 min.
Hydroquinone	UDPG	0.006	0.015	0.025
Hydroquinone	ADPG	0.011	0.023	0.045
Arbutin	UDPG	0.008	0.0125	0.020
Arbutin	ADPG	0.014	0.025	0.035

The system contained: 0.4  $\mu$ mole, hydroquinone or arbutin; 0.1  $\mu$ mole, cysteine; 0.1  $\mu$ mole, ethylenediamine tetraacetic acid; 20  $\mu$ moles *tris*(hydroxymethyl)aminomethane-maleate buffer (pH 6.8); 0.04 ml., wheat germ enzyme (fraction I). Total volume, 0.1 ml. The reaction mixture was incubated at 37° for the times indicated and the UDP or ADP formation was estimated (ref. 6).

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### Phage Ghost-induced Spheroplasts of *E. coli* 'B' as a System for Phage Reproductions

THE investigations of various authors have shown that lysozyme protoplasts, as well as penicillin spheroplasts, preserve the biochemical activity of an intact bacterium. Synthesis of proteins and nucleic acids is achieved in them<sup>1,2</sup>.

The full metabolic value of protoplasts allowed them to be used as a system for the study of the reproduction of phages.

In the work reported here, spheroplasts of *E. coli* B, obtained by means of treatment with ghosts of phage