

with leucine (Fig. 1). A study of the rate of reaction of N-ethylmaleimide with cysteine under the conditions employed here showed that the reaction goes to completion almost instantaneously. The N-ethylmaleimide is completely stable under acid conditions but should not be exposed, even momentarily, to alkaline conditions as it is converted to N-ethylmaleamic acid, which will then react with the ninhydrin reagent.

N-ethylmaleimide is used regularly in this laboratory to prevent the interference of sulphhydryl groups with the ninhydrin assay of proteolytic activity and has been found to give very good results under a wide variety of conditions.

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Chemical Oxidation of Uridine Diphosphate-Glucose to Uridine Diphosphate-Glucuronic Acid

THE importance of uronic acids in carbohydrate metabolism has only recently begun to be elucidated. The metabolism of inositol and ascorbate is known to involve uronic acid intermediates^{1,2}, and nucleotide-bound glucuronic acid has been implicated in hyaluronic acid synthesis³.

Uridine diphosphate (UDP)-glucuronic acid has been prepared chemically by Khorana and may also be produced enzymatically by the action of UDP-glucose dehydrogenase⁴. Both these preparations are relatively time-consuming and require the separation of the product from several contaminating reactants and side products.

Marsh has reported the oxidation of glucose-1-phosphate to glucuronic acid-1-phosphate⁵, and this report describes a similar oxidation of UDP-glucose.

Powdered platinum catalyst was prepared in aqueous solution from Adam's platinum oxide catalyst by hydrogenation at atmospheric pressure. 100 mgm. of reduced catalyst were suspended in 10 c.c. of water and introduced into a narrow vessel similar to that described by Marsh. The cylindrical vessel and sintered glass disk were attached to a U-shaped tube and immersed in a constant temperature bath; oxygen was bubbled through the solution at a rate adequate to keep the heavy catalyst particles in suspension. The UDP-glucose solution (63 μ moles) was then added, and oxygenation was allowed to proceed for 10½ hr. at a temperature of 55°, the pH being maintained between 7 and 8 by addition of 1 per cent potassium carbonate. This contrasted with the oxidation of glucose-1-phosphate at 45°, at which temperature no UDP-glucuronic acid was produced. At the end of the reaction the catalyst was removed by filtration, washed, and the filtrate and washings assayed for uronic acid content⁶.

In order to show that the uronic acid produced was still nucleotide-linked, an aliquot was spotted for paper electrophoresis in 0.1 M citrate-phosphate buffer, pH 2. The electrophoresis was carried out at 800 V. for 2 hr., and the two ultra-violet absorbing spots were eluted from the paper with water; one of them gave a positive carbazole test, uridine spectrum, and showed a 1:1 ratio of uronic acid to uridine based on a molar extinction coefficient for uridine of 10,000, the other material being unreacted UDP-glucose. The UDP-glucuronic acid could also be assayed by enzymatic transfer to *o*-aminophenol as described by Dutton and Storey, utilizing a fraction of rat liver homogenate sedimenting between 500g and 37,000g as the enzyme system⁷.

Use of 5 per cent palladium on charcoal as catalyst and oxygenation of the reaction mixture for 7 hr. resulted in no increase in the amount of uronic acid present, indicating that no oxidation had occurred.

Table 1. VARIATION OF YIELD WITH TIME

Time (hr.)	Yield* (per cent)
5.0	12
5.5	24
10.5	39

* Based on recovered UDP-glucose.

On the basis of recovered UDP-glucose, the best yield under the conditions studied has been 39 per cent. It is felt that the ease of isolation and relatively short reaction time required make the reaction feasible for the production of UDP-glucuronic acid.

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Incorporation of [1-¹⁴C]-Isopentenyl Pyrophosphate into Polyisoprene

THE occurrence of 3-methyl-but-3-enyl-1-pyrophosphate (*isopentenyl* pyrophosphate) as an intermediate in the enzymic synthesis of squalene from mevalonic acid, using yeast extracts, has been demonstrated by Bloch *et al.*^{1,2}, and by Lynen *et al.*³. The latter authors also quote an unpublished report by Arreguin that *isopentenyl* pyrophosphate can be incorporated into the rubber of *Taraxacum kok-saghyz* latex. The *in vitro* conversion of mevalonic acid lactone into polyisoprene in the presence of natural rubber (*Hevea brasiliensis*) latex has been shown by Park and Bonner⁴ and by Kekwick *et al.*⁵. In the experiments reported here it is demonstrated that *isopentenyl* pyrophosphate, on incubation with fresh *Hevea brasiliensis* latex, is rapidly converted into high molecular weight polyisoprene with essentially 100 per cent efficiency.

Fresh latex was obtained from the young branches of two 18-months-old specimens of *Hevea brasiliensis* (Clone P.B. 86) growing in the tropical greenhouse