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## A New Enzymatic Method for the Determination of Free and Conjugated Glucuronic Acid

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**Summary:** A new method is reported for the quantitative determination of glucuronic and galacturonic acid, which is based on the spectrophotometric measurement of NADH. The NAD-linked oxidation of the uronic acids to the corresponding dicarboxylic acids is measured in the presence of uronic acid dehydrogenase. This enzyme was isolated from *Pseudomonas syringae*. The test is highly specific for glucuronic and galacturonic acid and permits the exact determination of free and conjugated glucuronic acid. This enzymatic determination of glucuronic acid is the most sensitive method available today.

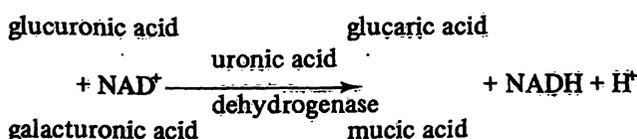
### Neue enzymatische Methode zur Bestimmung freier und konjugierter Glucuronsäure

**Zusammenfassung:** Es wurde eine neue Methode zur quantitativen Bestimmung von Glucuron- und Galakturonsäure mit Hilfe des optischen Tests entwickelt. Die NAD-gebundene Oxidation der Uronsäuren zu den entsprechenden Dicarbonsäuren wird in einer durch Uronsäure-Dehydrogenase katalysierten Reaktion gemessen. Das Enzym wurde aus *Pseudomonas syringae* isoliert. Der Test ist sehr spezifisch für Glucuron- und Galakturonsäure. Mit ihm kann freie und konjugierte Glucuronsäure genau bestimmt werden. Im Augenblick ist diese enzymatische Methode die empfindlichste Methode zur quantitativen Glucuronsäurebestimmung.

### Introduction

Methods as yet available for the determination of glucuronic acid are based on the carbazole reaction of *Dische* (1). Various modifications of this colour reaction with higher sensitivity were summarized by *Marsh* (2). These tests have certain disadvantages; they are not specific, and they are subject to interference by various compounds, they are also time-consuming, and they do not differentiate between free and conjugated glucuronic acid. A less sensitive method using gas chromatography was described by *Inoue* (3). Until now, a specific enzymatic method for glucuronic acid determination was lacking.

This paper describes a new method for the quantitative determination of glucuronic and galacturonic acid which is based on the NAD<sup>+</sup>-linked enzymatic oxidation of these uronic acids to the corresponding dicarboxylic acids with the stoichiometric formation of NADH:



The uronic acid dehydrogenase was discovered in the phytopathogenic bacterium *Pseudomonas syringae* (4) and purified and characterized (5, 6). The values ob-

tained are identical for both uronic acids. In the presence of both uronic acids the galacturonic acid content can be determined according to *Nagel* (7) and subtracted from the total content determined by the uronic acid dehydrogenase test. The main interest of this study was the development of a sensitive glucuronic acid assay for samples containing only free and/or conjugated glucuronic acid.

### Materials and Methods

*D*-glucuronic acid grade I (Sigma, St. Louis, Missouri, USA) and *D*-galacturonic acid (Serva, Heidelberg, FRG) may contain an unknown amount of water. Before use as standards they were dried to constant weight at 50°C under vacuum in a rotation evaporator. Phenolphthalein- $\beta$ -*D*-glucuronide sodium salt, and *p*-nitrophenyl- $\beta$ -*D*-glucuronide (Serva), testosterone- $\beta$ -*D*-glucuronide sodium salt, chromatographically pure was obtained from C. Funke, Düsseldorf, NAD<sup>+</sup> grade I, and  $\beta$ -glucuronidase from *E. coli* K 12 ( $\beta$ -*D*-Glucuronide glucuronosohydrolyase EC 3.2.1.31) about 100 kU/l (Boehringer, Mannheim, FRG). Uronic acid dehydrogenase (Uronate: NAD<sup>+</sup> 1-oxidoreductase EC 1.2.1.35) from *Pseudomonas syringae* ATCC 13394 was isolated from cells grown on glucuronic acid according to *Wagner* (6). For quantitative glucuronic acid determination, the enzyme fraction after Sephadex G-100 chromatography containing 25–30 kU/l was used. All other reagents were analytical grade (Merck, Darmstadt, FRG).