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This Week's Citation Classic

Dahlqvist A. Method for assay of intestinal disaccharidases.
Anal. Biochem. **7**:18-25, 1964. (University of Lund, Lund, Sweden)

The article describes a technically very simple method for the assay of disaccharidase activity with a TRIS-buffered glucose oxidase reagent. The method is so sensitive that it can be used for assay of the enzyme activities in peroral biopsy specimens of the small-intestinal mucosa and is thus useful for clinical studies in human subjects. [The *SCI*® indicates that this paper has been cited over 540 times since 1964.]

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"When this paper was written, I had studied the biochemistry of the small-intestinal disaccharidases for many years. Assay methods, based on the increase in reducing power during the hydrolysis of disaccharides, were used for these studies. With reducing disaccharides as substrates, e.g. maltose, isomaltose, and lactose, such methods have low sensitivity because a large fraction of the substrate has to be hydrolysed before the increase in reducing power can be measured with reasonable accuracy.

"At the time, recent findings of different groups of patients with impaired ability to digest disaccharides made it urgent to develop an accurate micromethod for disaccharidase activity which could be used for the analysis of such small amounts of tissue as would be obtained by peroral biopsy.

"It appeared obvious that replacement of reducing sugar methods with enzymatic assay of liberated glucose by glucose oxidase would yield considerable advantages. When different glucose oxidase preparations were tested, however, they were all found to contain strong contaminant disaccharidase activity, which prohibited their use.

"Considerable efforts were made to separate the glucose oxidase activity from these disaccharidase activities, but these attempts failed completely.

"The finding that the disaccharidase activities could be inhibited by TRIS, without any decrease in glucose oxidase activity, was made by pure chance. TRIS was not regarded as an enzyme inhibitor. In fact there was printed on the label of the TRIS bottle that it 'does not inhibit enzymes.' When I decided to look for an inhibitor for the disaccharidase activities which would not inhibit the glucose oxidase, I therefore tested numerous other substances than TRIS, without success. When for a certain experiment TRIS buffer was used instead of the usual buffer in order to avoid precipitation with other reagents, I found that TRIS had just the properties I had been looking for.

"The simple method for disaccharidase activity assay that was described in the paper has been used for numerous clinical investigations, and is now used in many hospitals as a routine method for clinical diagnosis in patients with intestinal disturbances.

"Few things are so good that they cannot be made better. It was later found that the method could be further simplified by using TRIS also to interrupt the first incubation step.[1] In this way one step of dilution, boiling, and pipettation could be omitted and at the same time a further tenfold increase in sensitivity was achieved."

REFERENCE

1. **Dahlqvist A.** Assay of intestinal disaccharidases. *Enzymol. Biol. Clin.* **11**:52-66, 1970.