MEASUREMENT OF INVERTASE, MALTASE AND β-GALACTOSIDASE ACTIVITIES ON SMALL SAMPLES OF INTESTINAL MUCOSA

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Recent discoveries on the absence of certain hydrolytic enzymes in human intestinal mucosa [1-4] have suggested a new type of molecular disease involving invertase, maltase and β-galactosidase (lactase). These enzymes have been partially isolated by Dahlquist in various animals. [5-7] Miller and Crane have demonstrated the presence of these enzymes in the brush border of intestinal epithelium. Recently a review on the localization of these activities in mucosa epithelial cells was presented by Dahlquist.[8]

The attempts to diagnose these deficiences are hindered by the lack of a quick and reliable micro method of assay on intestinal biopsy samples. Hence we are proposing the following method for this purpose. This method has the advantage of giving the worker the opportunity of working with very small samples of a biological specimen. This is extremely important if one desires to obtain information on small samples obtained by intestinal biopsy.[9] Usually one obtains about 2-3 mg. samples with such a technique. This is too small a sample for the chromatographic measurement of these activities. Furthermore, a chromatographic method yields only semiquantitative results. We are describing a method that obviates the above listed difficulties.

Material and Method

Reagents and equipment : ATP, TPN, EDTA, glucose oxidase, peroxidase, imidazole and o-dianisidine were obtained through the Sigma Chemical Company. Glucose-6-phosphate dehydrogenase was obtained through C. F. Boehringer. Yeast hexokinase was prepared according to the method of Kaplan and Colowick.[10].

Glucose, fructose, saccharose, lactose, maltose, magnesium chloride and potassium phosphate were commercial preparations.

Principle of Assay

$$\text{Glucose} + \text{ATP} \xrightarrow{Mg^{++}} \text{Glucose 6-Phosphate} + \text{ADP}$$

$$\text{Glucose 6-Phosphate} + \text{TPN} \longrightarrow \text{6-Phosphoglucunolactone} + \text{TPNH} + H^{+}$$

This so called glucose 6-phosphate coupled hexokinase system is used to measure hexokinase activity as well as glucose and glucose 6-phosphate content of tissues.[11]

Invertase activity is :

Saccharose + H_2O ⟶ β-D Fructose + α-D Glucose

Maltase activity is :

Maltose + H_2O ⟶ 2α-D-Glucose

Lactase activity is :

Lactose + H_2O ⟶ β-D-Galactose + α-D-Glucose

Hence the resulting α-D-Glucose molecule can be estimated by the hexokinase-glucose 6-phosphate dehydrogenase system. The O. D. followed at 340 mμ will give the TPNH formed. So by following the O. D. change at 340 mμ, one can estimate the invertase, maltase and lactase activities.

The preparation of hexokinase and glucose 6-phosphate dehydrogenase used were devoid of TPNH oxidase, glutathione reductase, phosphoglucose, isomerase, phosphoglucomutase, amylase, maltase, invertase and lactase.

Method

The assay mixture consisted of the below components for various activities :

Invertase : Imidazole at pH 7.5 10^{-1} M, EDTA 10^{-3} M, Mg^{++} 10^{-2} M, ATP 10^{-3} M TPN+ 2×10^{-4} M, sucrose 10^{-2} M

Maltase : Imidazole at pH 7.5 10^{-1} M, EDTA 10^{-3} M, Mg^{++} 10^{-2} M, ATP 10^{-3} M, TPN+ 2×10^{-4} M

Lactase : Imidazole at 7.5 10^{-1} M, EDTA 10^{-3} M, Mg^{++} 10^{-2} M, ATP 10^{-3} M, TPN+ 2×10^{-4} M, lactose 5×10^{-2} M (in certain assays 10^{-2} M was used).

Hexokinase and glucose 6-phosphate dehydrogenase were added in sufficient amounts (about 100 times more than the activity assessed).

The control experiments were carried on with intestinal mucosa of various experimental animals. The mucosa was scraped with a glass slide and was homogenized in a Waring Blendor with 5×10^{-3} M pH 7.5 EDTA. The resulting homogenate was used for the assay.

The biopsy specimens, obtained according to the method of Crosby and Kugler,[9] were frozen in dry ice and kept frozen. At the time of assay they were homogenized in a sintered walled all-glass homogenizer and the homogenate was used for the assay.

Various amounts of mucosal homogenate were used in control experiments. However, for biopsy specimens as little as 0.07 - 0.1 mg.

mucosa, per ml. of incubation mixture was enough to obtain a satisfactory result. As much as 0.4 - 0.5 mg. mucosa per ml. of incubation mixture can be used.

Protein content of the homogenate was estimated according to the method described by Colowick.[12]

At times the results were run in duplicate and determinations were carried on with glucose oxidase.[13] This method is complicated by the presence of invertase activity in the commercial preparations of glucose oxidase. This difficulty is obviated by the use of TRIS as an inhibitor in these assays.

The activities were expressed as micromoles of dissaccharide hydrolized per miligram of protein content of biopsy specimen per hour.

The assay was conducted at room temperature which varied between 25 - 27° C.

Results

The sensitivity of the method is described in the following experiment.

Figure 1 shows the chromatography of the incubation mixture after 0, 1, 2, 3, 6 hours of incubation. As can be seen, there is very little glucose and fructose present even after 6 hours of incubation, when such a small amount of intestinal mucosa is used for the dissaccharidase assay.

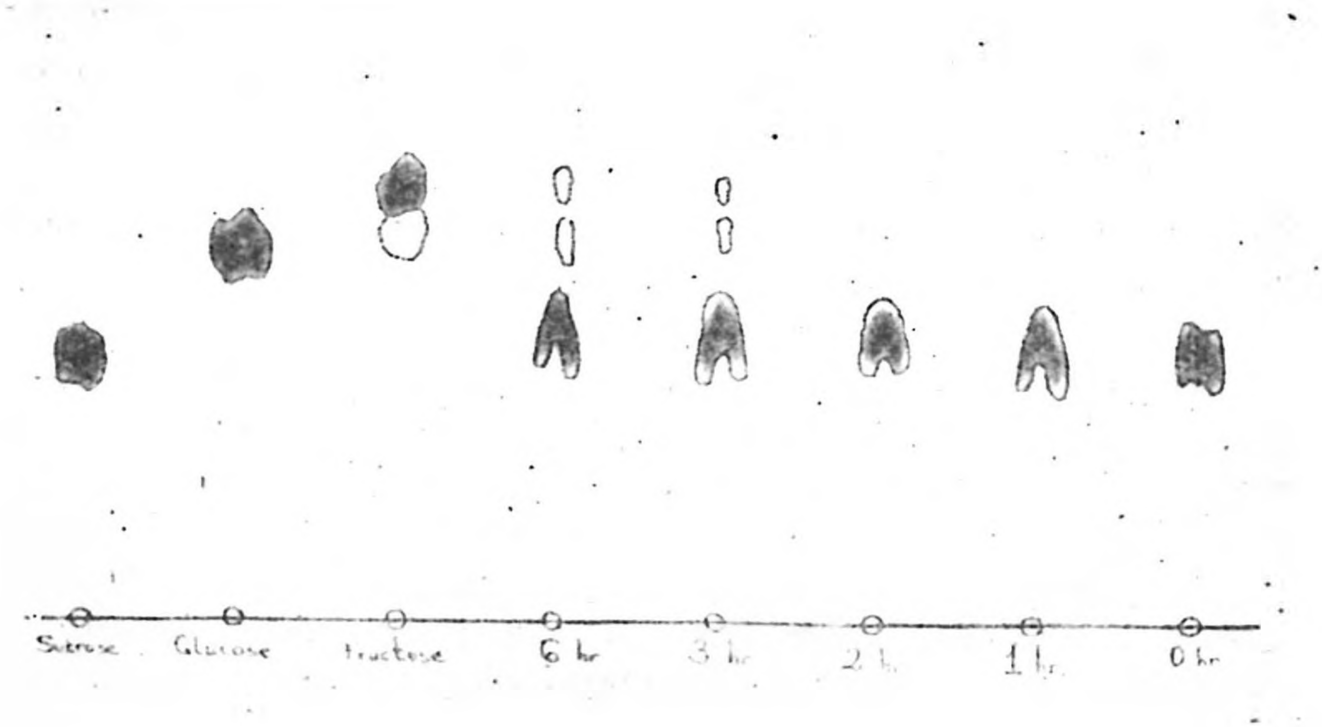

Figure 1. Assay mixture was the same as described in methodology. Pig intestinal mucosa was used and 0.06 mg. mucosal protein was present per cc. of incubation mixture.

The same assay mixture was used for spectrophotometric determination on invertase, lactase and maltase and gave the results listed in Table 1.

Following controls were carried on.

TPNH oxidation : The rate of TPNH oxidized by various homogenates were found to be negligible (Table 2).

As can be seen from Table 2, although TPNH is oxidized it is not at a rate to alter the invertase and maltase activities to a considerable extent.

TABLE 1

THE SPECTROPHOTOMETRIC ASSAY OF INVERTASE, LACTASE AND MALTASE. PIG INTESTINAL MUCOSA IS USED. 0.06 MG. MUCOSAL PROTEIN WAS PRESENT PER ML. OF INCUBATION MIXTURE

		O. D. increase per minute	Unit of activity
Pig*	Invertase	0.003	0.49
	Maltase	0.013	1.05
Beef	Invertase	0.001	0.04
	Maltase	0.004	0.16
Sheep	Invertase	0.001	0.08
	Maltase	0.010	0.41
Dog	Lactase	0.001	0.03

* Results checked with glucose oxidase assay system.

TABLE 2

TPNH OXIDATION BY VARIOUS HOMOGENATES IMIDAZOLE AT PH 7.5 10^{-1} M, TPNH 10^{-4} M, EDTA 10^{-3} M. HOMOGENATES WERE ADDED TO 0.06 - 0.2 MG. MUCOSAL PROTEIN PER ML. INCUBATION MIXTURE

Homogenate	Decrease in TPNH O. D. in 20'	Increase at 340 $m\mu$ in invertase, lactase activities in 20'
Pig	0.012	0.060 - 0.260
Sheep	0.010	0.060 - 0.200

Fructose is known to be phosphorylated by hexokinase. Slowing down of the increase at 340 $m\mu$ was observed in invertase assay after 30 minutes of incubation and that this was due to fructose accumulation was confirmed by adding known amounts of fructose to the incubation mixture at the beginning and measuring the initial rates.

This slowing down was not observed with maltase.

Glutathione reductase was absent in the enzyme system used

and the oxidized glutathione present in the incubation mixture was too low to complicate the assay.

The spectrophotometric assay was controlled as follows.

Two identical maltose incubation mixtures were prepared; one of them was followed spectrophotometrically. The other one had no hexokinase, ATP, TPN or glucose 6-phosphate dehydrogenase. In the spectrophotometrically assayed cuvette maltase split at 20′, 40′ and 60th minutes were calculated. In the other cuvette where no hexokinase, ATP, TPN or glucose 6-phosphate dehydrogenase were present, aliquots were drawn at 20′, 40′ and 60th minutes and were treated with equal amounts of 1 N perchloric acid. The supernatant was neutralized with KOH and glucose was estimated again with the hexokinase system.[11] The results are shown in Table 3

Preliminary results obtained on human intestinal biopsy samples :

TABLE 3

SPECTROPHOTOMETRIC ASSAY VERSUS GLUCOSE DETERMINATION
(Details in the text. All values are reported as units for 1 hour.)

	Units calculated for spectrophotometric assay depending on μ mole maltase split	Units calculated for glucose production depending on glucose μ mole produced
For 20' assay	1.86	1.70
For 40' assay	1.95	1.80
For 60' assay	1.83	1.55

Some of the results obtained on 4 human intestinal biopsies are shown in Table 4. One of them was a normal subject, three were suspected of dissaccharidase deficiency and will be published separately.

TABLE 4

DISSACCHARIDASE ACTIVITIES IN HUMAN INTESTINAL MUCOSA
(Details reported in text.)

Subject	Biopsy specimen mg.	Invertase unit	Maltase unit	Lactase unit
Normal	3.29	0.07	0.27	0.07
Ş. K.	3.1	0.00	0.00	0.00
S. A.	2.1	0.03	0.15	0.06
H. Ç.	8.43	0.032	0.00	0.00

Discussion

This reported method is a quick and reliable semiquantitative assay for various dissaccharidases on small samples of biological specimens. Preliminary results on four human intestinal biopsy specimens are presented.

Summary

A micro method for the determination of invertase, maltase, and lactase is described.

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