

HUMAN INTESTINAL DISACCHARIDASES AND HEREDITARY
DISACCHARIDE INTOLERANCE. THE HYDROLYSIS OF
SUCROSE, ISOMALTOSE, PALATINOSE (ISOMALTU-
LOSE), AND A 1,6- α -OLIGOSACCHARIDE (ISO-
MALTO-OLIGOSACCHARIDE)
PREPARATION *

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In recent investigations, the specificity of the human intestinal disaccharidases has been studied and their relation to different forms of hereditary disaccharide intolerance discussed (1, 2). The results have indicated isomaltose and sucrose to be hydrolyzed by two different enzymes in the human intestine.

In inborn errors of metabolism, as far as we now know, only a single enzyme is missing. In several patients with sucrose intolerance, a certain intolerance for starch and maltose-dextrin mixtures has also been described (3). After the administration to such patients of a 1,6- α -oligosaccharide preparation containing chiefly isomaltose, severe intolerance symptoms occurred (4). This was taken to indicate that the patients had intolerance for both sucrose and isomaltose, and thus should lack two different enzyme activities. The patients also had intolerance for palatinose (isomaltulose), a sugar with a close structural resemblance to isomaltose (3, 4).

The aim of the present investigation was to study further the relation between the isomaltase and invertase (sucrase) activities of human small intestinal mucosal preparations, and also the importance of these enzymes for the hydrolysis of palatinose and the 1,6- α -oligosaccharide prepara-

tion used for the tolerance tests. Both heat inactivation experiments under varying conditions and mixed-substrate incubations have been performed.

MATERIALS AND METHODS

Mucosal preparations. Macroscopically normal pieces of small intestine removed from adult humans during abdominal operations were used. The mucosa was removed with a piece of glass and homogenized with 4 vol water or saline in an Ultra-Turrax homogenizer while the tube was chilled with crushed ice. After centrifugation at about $1,000 \times g$ for 10 minutes, the opalescent supernatant fluid was used for the experiments.

Disaccharides. Maltose¹ and sucrose² were obtained commercially. Palatinose³ was received as a gift, as was isomaltose,⁴ which was also prepared in the laboratory as previously described (5).

1,6- α -Oligosaccharide preparations. These were prepared by the enzymic hydrolysis of dextran as described previously (4). The preparations contained isomaltose, isomaltotriose, and higher isomalto-oligosaccharides. The average degree of polymerization of the lots used in the present investigation varied between 2.4 and 3.4.

Disaccharidase activity was measured by the method of Dahlqvist (6). In some of the experiments, maleate buffer of pH 6.5 was used. The optimal pH for the human enzymes is, however, somewhat lower than for the pig enzymes (7); in some of the experiments, therefore, maleate buffer of pH 5.8 was used instead. The difference in activity at the two pH values is small. The

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¹ 4-(α -D-Glucosido)-D-glucose, monohydrate; Pfanstiehl Laboratories, Inc., Waukegan, Ill.

² 2-(α -D-Glucosido)- β -D-fructofuranoside; Baker Chemical Co., Phillipsburg, N. J.

³ 6-(α -D-Glucosido)-D-fructose, monohydrate; generously given by Dr. R. Weidenhagen, Neuoffstein, Germany.

⁴ 6-(α -D-Glucosido)-D-glucose; generously given by Dr. R. Weidenhagen, Neuoffstein, Germany.

activity liberating 1 mg of monosaccharides in 60 minutes at 37° C at 0.028 M substrate concentration has been used as the unit of disaccharidase activity.

1,6- α -Oligosaccharidase activity. For the determination of the 1,6- α -oligosaccharidase activity, the reaction mixture contained 9.5 mg per ml of the 1,6- α -oligosaccharide preparation instead of disaccharide. Incubation conditions and assay of the glucose liberated were the same as for the disaccharidases. Since the average degree of polymerization of the 1,6- α -oligosaccharide preparations was somewhat higher than 2, the concentration of sugar during incubation with this substrate was somewhat lower than 0.028 M. The activity liberating 1 mg of glucose in 60 minutes at 37° C was used as the unit of activity. In addition to glucose, some oligosaccharides were formed from the trisaccharide and higher polymers present. These were not measured with the Tris-glucose oxidase reagent.

The separate assay of fructose and glucose present in mixture has recently been described (8). This method is based upon the reaction of glucose with a hexokinase: ATP:glucose 6-phosphate dehydrogenase:TPN system to yield TPNH₂, which is measured spectrophotometrically. When the glucose present has reacted, phosphohexose isomerase is added, and fructose also reacts. The difficulty with this method is that the enzyme preparations used contain traces of phosphohexose isomerase, so that the limit between the amounts of glucose and fructose present must be obtained by extrapolation (8). We found it difficult to obtain reproducible values with this method, and preferred to use a modification in which the amount of glucose present was determined with the Tris-glucose oxidase reagent, and the sum of glucose and fructose with the enzyme system described above.

a) Assay of glucose. A sample of the solution to be analyzed containing 5 to 50 μ g of glucose was diluted with water to 0.5 ml. After the addition of 3.0 ml of Tris-glucose oxidase reagent (6), the tube was incubated at 37° C for 1 hour. Then the color produced was measured in a Beckman B spectrophotometer at 420 m μ or in an Eppendorf photometer with filter 436, using 1-cm light-path cuvettes.

b) Assay of glucose and fructose. An enzymic reagent⁵ was prepared by mixing: 62 ml 0.05 M triethanolamine buffer at pH 7.6, 2 ml 0.1 M MgCl₂, 2 ml ATP solution at 51.4 mg per ml, 1 ml hexokinase solution at 2 mg per ml, 1 ml glucose 6-phosphate dehydrogenase solution at 2 mg per ml, and 1 ml phosphohexose isomerase solution at 2 mg per ml. The reagent was stable for at least a few days in the refrigerator.

A sample of the solution to be analyzed containing 10 to 50 μ g of glucose and fructose was poured into a 2-cm light-path quartz cuvette, diluted with water to 0.5 ml, and then 3.5 ml of the enzymic reagent was added. After mixing with a glass rod, the cuvette was put into

an Eppendorf photometer with filter 336, and the apparatus was adjusted to show zero extinction. Then 0.1 ml of a solution of TPN at 5 mg per ml in distilled water was added to start the reaction. The increase in optical density was followed until the reaction was complete, which took 3 to 8 minutes, depending on the amount of sugar present.

The amount of glucose and fructose present was calculated from a standard curve obtained with glucose or fructose. The two monosaccharides yielded identical standard curves. The amount of fructose present was calculated as the difference between the amount of glucose and fructose found by this method and the amount of glucose found with the Tris-glucose oxidase reagent. In analyzing solutions with known amounts of glucose and fructose, the results obtained by this method agreed well with theory. Isomaltose and sucrose did not interfere with the reaction.

Protein was assayed by the method of Lowry, Rosebrough, Farr, and Randall (9) with the modified reagent B introduced by Eggstein and Kreutz (10). A standard curve was prepared with human serum albumin.⁶

Heat inactivation experiments were performed as described earlier (11).

Mixed-substrate incubations are valuable for the evaluation of whether two substrates acted upon by an enzyme preparation are attacked by the same or by two different enzymatically active centers. In the present investigation, the performance and the calculation of the results of these incubations varied with whether the same or different hydrolytic products were formed from the two substrates under consideration.

a) Different hydrolysis products from the two substrates. With sucrose-isomaltose and palatinose-isomaltose mixtures, one of the substrates yields an equimolar mixture of glucose and fructose, whereas the other yields only glucose. It was therefore possible to calculate the rate of hydrolysis of each substrate separately. In these cases, enzyme kinetic analysis was performed by the method of Lineweaver and Burk (12) with the modification introduced by Dixon (13). To ensure that zero-order kinetics was obeyed, care was taken in these experiments that the hydrolysis of the substrate or the inhibitor never exceeded 15%.

First, a series of different concentrations of one of the substrates alone was incubated with a constant amount of the enzyme preparation, and the substrate constant K_s for this substrate was calculated. Then the same experiment was performed in the presence of a constant amount of the other substrate; if inhibition occurred, the kind of inhibition (competitive or noncompetitive) was noted and the inhibitor constant K_i calculated (see Figure 4). When two substrates are acted upon by the same enzymatic center, they will act as competitive inhibitors for each other, and the value of K_i for one substrate as the competitive inhibitor of the other will numerically equal the K_s for the first substrate itself as a substrate for the enzyme.

⁵ All the enzyme and coenzyme preparations were obtained from Boehringer & Soehne A. G., Mannheim, Germany.

⁶ Kindly supplied by Kabi AB, Stockholm, Sweden.

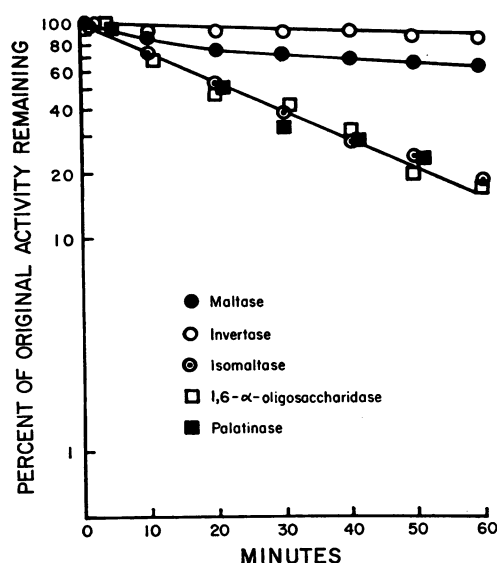


FIG. 1. HEAT INACTIVATION OF A HOMOGENATE OF HUMAN SMALL INTESTINAL MUCOSA AT 46° C IN 0.01 M SODIUM PHOSPHATE BUFFER AT pH 7.0. The preparation was obtained from the jejunum. The solution contained 2.4 mg protein, 15.0 U maltase, 4.5 U invertase, 4.5 U isomaltase, 0.9 U palatinase, and 4.1 U 1,6- α -oligosaccharidase activity per milliliter (units defined in Materials and Methods).

b) The same hydrolytic product from the two substrates. With mixtures of sucrose-palatinose and isomaltose-1,6- α -oligosaccharide preparation, both substrates yield the same products, and the determination of K_i is then impossible by the methods used here. In these cases, the amounts of glucose liberated from each of the substrates alone and from a mixture of the two substrates were compared.

RESULTS

Heat inactivation

Heat inactivation experiments were performed at different temperatures in buffers of different pH. Figure 1 shows the course of heat inactivation at pH 7.0 at 46° C and Figure 2, that at pH 5.0, 7.0, and 8.0 at 50° C.

The separation of the invertase and isomaltase activities described earlier (1) was verified. Under all the conditions tested, the isomaltase activity was inactivated much more rapidly than the invertase. In the experiment shown in Figure 1, in 1 hour more than 80% of the isomaltase activity was inactivated, but only 15% of the invertase activity. When the same solution was heated at 45° C instead of 46° C, 70% of the isomaltase ac-

tivity was inactivated in 1 hour, while the invertase activity was not affected at all. As in Figure 2, on the other hand, when heating was performed at 50° C, in 10 minutes at pH 7.0 more than 90% of the isomaltase was inactivated and only 25% of the invertase. The intestinal invertase and isomaltase activities were thus completely separated from each other.

The course of inactivation of the maltase activity in all cases agreed well with the proposal made previously, that 40 to 50% of the total maltase activity is exerted by the same enzyme as exerts the isomaltase activity (1).

The inactivation of the palatinase and 1,6- α -oligosaccharidase activities in all the experiments ran parallel with the inactivation of the isomaltase

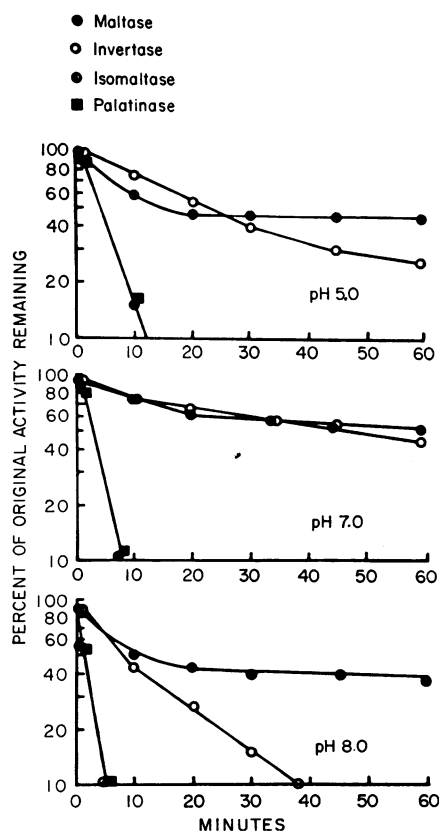


FIG. 2. HEAT INACTIVATION OF A HOMOGENATE OF HUMAN SMALL INTESTINAL MUCOSA AT 50° C IN DIFFERENT BUFFERS: 0.01 M SODIUM ACETATE AT pH 5.0, 0.01 M SODIUM PHOSPHATE AT pH 7.0, AND 0.01 M SODIUM BARBITAL AT pH 8.0. The preparation was obtained from the ileum. The solution contained 2.0 mg protein, 16.0 U maltase, 4.0 U invertase, 3.0 U isomaltase, and 1.0 U palatinase per milliliter (units defined in Materials and Methods).

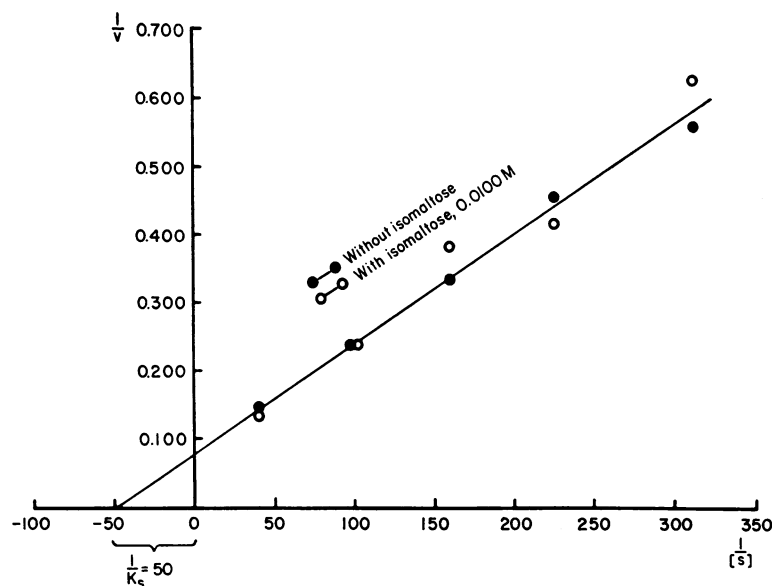


FIG. 3. LINEWEAVER-BURK-DIXON PLOT OF INVERTASE ACTIVITY WITH (0.0100 M) AND WITHOUT ISOMALTOSE. Isomaltose does not inhibit intestinal invertase. v = rate of hydrolysis of sucrose and $[s]$ = concentration of substrate (sucrose) in moles per liter.

activity, indicating all these three activities to be caused by a single enzyme.

The rate of inactivation of all these activities at a certain temperature and in a certain buffer varied to some extent in different preparations (e.g., compare Figure 1 here with Figure 1 in reference 1). Their heat sensitivity relative to each other did not vary, however. The small variations in rate of inactivation probably were caused by the different purity of preparations.

Mixed-substrate incubations

Isomaltose and sucrose in mixture. Isomaltose and sucrose did not inhibit each other's hydrolysis, but were hydrolyzed completely independently (Figure 3, Table I). This indicates the intestinal isomaltase and invertase to be two different enzymes. The K_s for the isomaltase is 6.75×10^{-3} and for the invertase, 1.8×10^{-2} .

Isomaltose and palatinose in mixture. Palatinose inhibited the isomaltase activity competitively, with a K_i that was calculated to be 6.5×10^{-3} (Figure 4). The K_s for palatinose as substrate in other experiments was found to be 6.1×10^{-3} . The good agreement between the K_s and K_i values strongly indicates that palatinose and isomaltose are hydrolyzed by the same enzyme. This

enzyme seems to have the same affinity for the two substrates, but palatinose is hydrolyzed 25 to 30% as fast as isomaltose. Isomaltose could not be used as an inhibitor for the palatinase activity,

TABLE I
The hydrolysis of sucrose and isomaltose separately and in mixture*

Sugar concentration		Glucose liberated in 0.25 ml of reaction mixture	Theoretical amount of glucose†
Sucrose	Isomaltose		
moles/L	moles/L	μg	μg
0.0250		34	
0.0100		21	
0.0063		15	
0.0045		11	
0.0033		9	
	0.0100	47	
	0.0100	47	
	0.0100	46	
0.0250	0.0100	78	81
0.0100	0.0100	66	68
0.0063	0.0100	64	62
0.0045	0.0100	60	58
0.0033	0.0100	58	56

* The amount of glucose formed from the two substrates in mixture equals the sum of the amounts formed with the substrates in different tubes. Thus the two substrates do not inhibit each other's hydrolysis.

† On the assumption of hydrolysis of isomaltose and sucrose by two different enzymes.

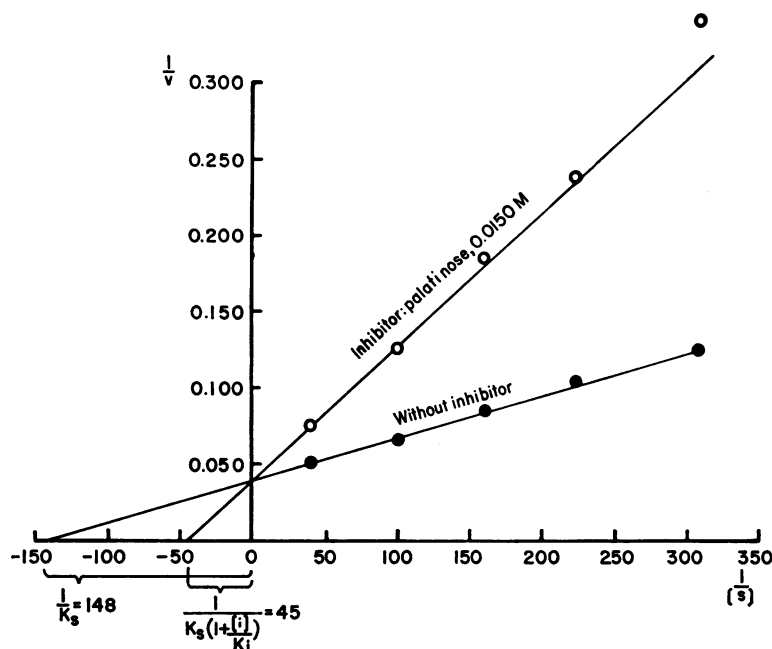


FIG. 4. LINEWEAVER-BURK-DIXON PLOT OF THE ISOMALTASE ACTIVITY WITH (0.0150 M) AND WITHOUT PALATINOSE. The substrate constant K_s for isomaltase activity was calculated at 6.75×10^{-3} . Palatinose inhibits isomaltase activity competitively, with the inhibitor constant K_i calculated to be 6.5×10^{-3} . This agrees well with the K_s for palatinose as substrate, which in other experiments has been calculated to be 6.1×10^{-3} . v = rate of hydrolysis of isomaltose, $[s]$ = concentration of substrate (isomaltose) in moles per liter and $[i]$ = concentration of inhibitor (palatinose) in moles per liter.

since the inhibitor would have been too rapidly hydrolyzed.

Sucrose and palatinose in mixture. The hydrolysis of sucrose and palatinose separately and in mixture is seen in Table II. The amount of

glucose formed in 0.014 M sucrose and 0.014 M palatinose in mixture is 95% of the sum of the amounts formed from each of the two disaccharides in separate tubes. This clearly indicates that sucrose and palatinose are hydrolyzed independently.

TABLE II

*The hydrolysis of palatinose and sucrose separately and in mixture**

Substrate	Glucose liberated in 0.1 ml of reaction mixture
	μg
0.028 M Palatinose	5.0
0.014 M Palatinose	5.5
0.014 M Palatinose + 0.014 M sucrose	25.7
0.014 M Sucrose	21.5
0.028 M Sucrose	32.3

* Each value is the mean of three separate determinations with good agreement among them. The results indicate that sucrose and palatinose are hydrolyzed independently of each other.

TABLE III

*The hydrolysis of isomaltose and a 1,6- α -oligosaccharide preparation separately and in mixture**

Substrate	Glucose liberated in 0.1 ml of reaction mixture
	μg
0.028 M Isomaltose	32.5
0.014 M Isomaltose	25.0
0.014 M Isomaltose + 4.8 mg per ml of 1,6- α -oligosaccharides	27.0
4.8 mg per ml of 1,6- α -oligosaccharides	17.3
9.5 mg per ml of 1,6- α -oligosaccharides	22.3

* Each value is the mean of three separate determinations with good agreement among them. The results fit with the theory that the two substrates are hydrolyzed at the same enzymatic center.

Isomaltose and the 1,6- α -oligosaccharide preparation in mixture. The hydrolysis of isomaltose and the 1,6- α -oligosaccharide preparation separately and in mixture is seen in Table III. The 1,6- α -oligosaccharide preparation used in this experiment had an average degree of polymerization of 3.4. The liberation of glucose from the 1,6- α -oligosaccharide preparation alone was somewhat lower than from isomaltose. From the two substrates in mixture, the amount of glucose liberated was 64% of the sum of the amounts formed from the two substrates in separate tubes. The results indicate that isomaltose and the 1,6- α -oligosaccharide preparation inhibit each other's hydrolysis and agree well with the theory that they are hydrolyzed by the same enzyme.

DISCUSSION

Both the heat inactivation experiments and the mixed-substrate incubations performed in this investigation have clearly demonstrated that the human intestinal isomaltase and invertase activities are exerted by two different, enzymatically active centers, thus confirming the results of an earlier investigation on this subject (1).

It has also been demonstrated that palatinose and the 1,6- α -oligosaccharide preparation previously used for clinical tolerance tests (3, 4) are hydrolyzed at the same enzymatic center as isomaltose. These results in turn confirm that the sucrose-intolerant patients who showed intolerance for the 1,6- α -oligosaccharide preparation in fact had an isomaltase deficiency in addition to their invertase deficiency (3, 4). Their intolerance for palatinose also is explained by the isomaltase deficiency.

Clinical observations made by other authors also indicate that isomaltose and sucrose intolerance occur together. A low tolerance for starch and dextrins has been observed in several sucrose-intolerant patients, in spite of their normal tolerance for maltose and the normal amylase activity in the intestinal contents (14-18). Intolerance for palatinose has also been demonstrated in some of these cases (16, 17). Furthermore, homogenates of mucosal biopsy specimens from patients with sucrose intolerance were unable to hydrolyze both sucrose and isomaltose *in vitro*, although these preparations rapidly hydrolyzed lactose and maltose (15).

This is remarkable, since the data reported in the present investigation indicate the human invertase and isomaltase to be two independent activities, which should mean that they are exerted by two different enzymes, although all inborn errors of metabolism previously investigated appear to be caused by the absence of a single enzyme. This indicates some kind of close relationship between the two disaccharidases.

Other facts, too, indicate a close relationship between isomaltase and invertase. In homogenates of human intestinal mucosa, a rather constant proportion was found to exist between the isomaltase and invertase activities, although the different samples obtained from different subjects had widely varying activity per milligram of protein or mucosa. The two activities are obtained in a common peak in gel filtration chromatograms (2, 19) even though several other peaks of disaccharidase activity are separated with this method. This points to the possibility either that the two activities are exerted by two enzymes that are rather similar proteins whose formation may be partly controlled by a common gene, or possibly that there is a single protein with two different, enzymatically active centers. Whether such a hypothetical protein should be counted as one or two enzymes is a matter of definition.

In the gel filtration chromatograms, an additional small invertase peak was found that moved more rapidly than the common invertase-isomaltase peak. The two invertase fractions had similar sensitivity to heat. The implications of this second invertase for hereditary disaccharide intolerance cannot at present be discussed.

Hitherto no indication has been found that in the human intestine more than one enzyme hydrolyzes isomaltose. This is in contrast with the pig, where the isomaltase activity is chiefly exerted by the (specific) isomaltase, but to some extent (around 10 to 15% of the total) by two other enzymes (20).

The hydrolysis of palatinose in the human intestine also seems to be catalyzed only by isomaltase. In the pig, on the other hand, this enzyme accounted for only about 35% of the total palatinase activity (20). This implies that in man, intolerance for palatinose seems a justifiable criterion for the lack of intestinal isomaltase. It should be observed, however, that palatinose is hydrolyzed

more slowly than isomaltose, and thus a decreased isomaltase activity in the small intestinal mucosa can result in intolerance for palatinose, although the activity remaining is sufficient to hydrolyze an administered mixture of 1,6- α -oligosaccharides (isomalto-oligosaccharides) (4).

A more detailed discussion of the enzymatic defect in patients with intolerance for sucrose and isomaltose must await further studies of the enzymes of the intestinal mucosa, both in these patients and in normal humans.

SUMMARY

1) The specificity of the human, small intestinal enzymes hydrolyzing isomaltose, sucrose, palatinose (isomaltulose) and a 1,6- α -oligosaccharide (isomalto-oligosaccharide) preparation have been studied with heat inactivation and mixed-substrate incubation experiments. 2) The isomaltase and invertase activities are independent of each other as shown by these methods. 3) Isomaltase is responsible for the hydrolysis of both palatinose and the 1,6- α -oligosaccharide preparation. The use of these for clinical tolerance tests to reveal intestinal isomaltase deficiency thus is justified. 4) The clinical investigations hitherto made indicate that hereditary intolerance for sucrose and isomaltose often, and possibly always, follow each other. These patients may lack two enzymes, which is remarkable in view of our previous knowledge of inborn errors of metabolism.

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