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Intestinal β -Galactosidases

II. BIOCHEMICAL ALTERATION IN HUMAN LACTASE DEFICIENCY

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ABSTRACT Despite the high prevalence of intestinal lactase deficiency in some racial groups and in patients with intestinal disease, the biochemical defect has not been characterized.

In the preceding paper normal intestine was found to have two lactases with distinctly different pH optima. Therefore, pH activity curves of homogenates from lactase-deficient intestine were studied, and the pH optimum was found to be shifted from the normal of 5.8 to 4.8. Density gradient ultracentrifugation of intestinal material from five lactase-deficient patients demonstrated absence of a lactase with pH optimum 6.0 and molecular weight 280,000. A second lactase with pH optimum 4.5 and molecular weights of 156,000 and 660,000 remained at normal levels accounting for the shift in the pH optimum in whole intestinal homogenates. In addition, three of the five patients had absence of a smaller β -galactosidase (molecular weight 80,000) that had specificity only for synthetic substrates. Although not a lactase, this enzyme had a pH optimum identical with the missing lactase, and its activity was inhibited by lactose in a partially competitive manner suggesting that it is capable of binding lactose. It is possible that this enzyme is a precursor or fragment of the missing lactase.

The residual lactase activity provided by the lactase with low pH optimum represents 20–70% of the activity of the missing enzyme, and yet these patients are not able to digest dietary lactose. Thus it appears that the residual enzyme plays no significant role in the hydrolysis of ingested lactose.

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INTRODUCTION

Despite the high incidence of lactase deficiency in healthy members of several racial groups (1–3) and in patients with active intestinal disease (4–7), previous studies utilizing crude intestinal homogenates have merely demonstrated a depression of enzyme activity, the biochemical defect having not been characterized. In a single patient with lactase deficiency, the pH activity curve for lactase in intestinal homogenates was said to be normal (8). Patients with low intestinal lactase secondary to malabsorption syndromes were later shown to have normal intestinal β -galactosidase activity with the synthetic substrate 6-bromo-2-naphthyl- β -galactoside (BNG) (9), suggesting that a second β -galactosidase remains at normal levels in lactase deficiency. Whether this second enzyme possesses lactase activity is unknown. More recently Cook and Dahlqvist (10) have found from studies on crude intestinal homogenates that enzyme activity at pH 5.5 against BNG is actually decreased in lactase-deficient Bantu patients. Presumably only “brush-border” lactase is deficient in these patients. Whatever the explanation for the depression of BNGase activity, it is apparent from our studies (12) that BNG is hydrolyzed by two different intestinal enzymes rather than by a single β -galactosidase (11), making it very difficult or impossible to draw conclusions from substrate specificity studies (9, 10) in which whole intestinal homogenates are used. Biochemical separation of the β -galactosidases is a prerequisite for meaningful study of the defect in lactase deficiency.

Since each of the two intestinal lactases described in the preceding paper has its own typical pH activity curve (12), loss of a single enzyme in lactase deficiency would be expected to cause a shift in the pH optimum to that of the remaining enzyme.

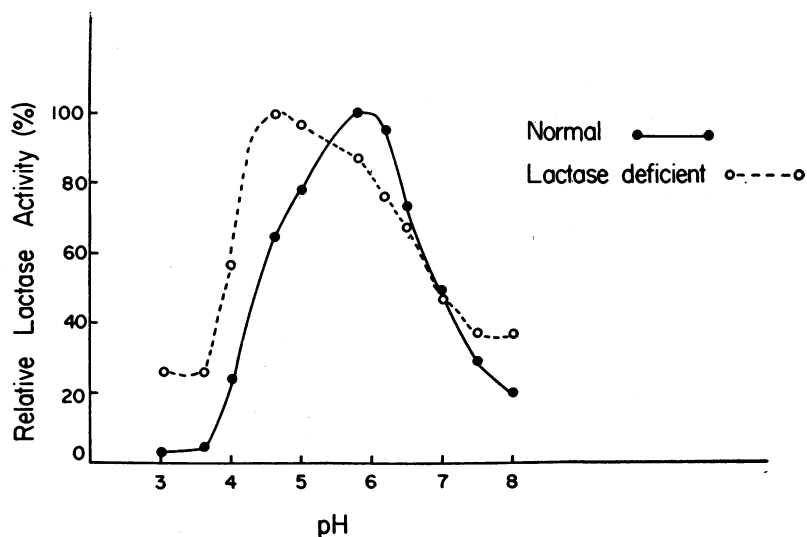


FIGURE 1 pH activity relationships for pooled intestinal homogenates from 10 normal and 10 lactase-deficient patients. See text for buffers.

We initiated the present work in the Tropical Research Laboratory where all adult patients with tropical sprue (5, 13) and many children with nonspecific intestinal disease¹ or parasitoses¹ have lactase deficiency. Biopsy material from these patients was used for pH activity studies, and subsequently density gradient ultracentrifugation was utilized to determine the biochemical abnormality in patients with lactase deficiency.

METHODS

Intestinal tissue was obtained at or just beyond the ligament of Treitz by use of the Crosby capsule (14) after verification by X-ray. The tissue was wrapped in Parafilm (Para Mfg. Co., Cranford, N. J.) and stored at -20°C to prevent loss of weight or enzyme activity (15).

Enzyme assays. After rapid thawing and homogenization of the tissue in 2.0 ml of cold 154 mM NaCl, 6-bromo-2-naphthyl- β -galactosidase (BNGase), nitrophenyl β -galactosidase, and lactase activities were assayed according to the semimicro methods outlined in the preceding paper (12).

pH activity studies were carried out as described in the previous paper (12). Assays, including substrate-enzyme blanks, were determined at a minimum of 10 different points on the pH curve.

Density gradient ultracentrifugation was used to study the sedimentation pattern of the β -galactosidases. Biopsies from four normal and five lactase-deficient patients were studied by this technique. Patients were considered to be lactase-deficient when they fulfilled the enzymatic and clinical criteria previously outlined (13). Biopsy tissue (15–30 mg) was homogenized in 5 ml of 0.01 M sodium-potassium phosphate buffer, pH 5.5, containing 154 mM NaCl and centrifuged at 100,000 g for 1 hr. The supernatant was recovered, concentrated in a dialysis bag overnight under a vacuum, and

the final 0.2 ml was dialyzed against 0.01 M sodium-potassium phosphate buffer, pH 5.5, with several buffer changes. All preparative steps were carried out at 4°C . 0.1 ml was then placed on a 5 ml density gradient at 5–20% NaCl in the same buffer and centrifuged at 158,000 g for 9 hr in the SW 50L rotor (Beckman Instruments, Inc., Palo Alto, Calif.) (12). The particulate formed upon centrifugation of the whole homogenate was solubilized by activated papain (12) and then prepared and studied by ultracentrifugation in the same way as the supernatant. In some experiments, centrifugation was carried out for only 2 hr to study the large molecular weight form of one of the enzymes (12).

TABLE I
Optimal pH in Lactase-Deficient Intestine*

Group	Total No.	pH of peak activity		
		4.8	5.8	Minor peak at 6.5†
Adults				
Tropical sprue	6	6	0	3
Idiopathic lactase deficiency	1	1	0	0
Totals	7	7	0	3
Children				
Tropical sprue	2	1	1	0
Trichuriasis	2	2	0	0
Idiopathic lactase deficiency	2	2	0	2
Totals	6	5	1	2

* pH optimum of normal intestinal homogenates = 5.8.

† Less than 50% of peak at 4.8.

¹ Genel, M. Unpublished observations.

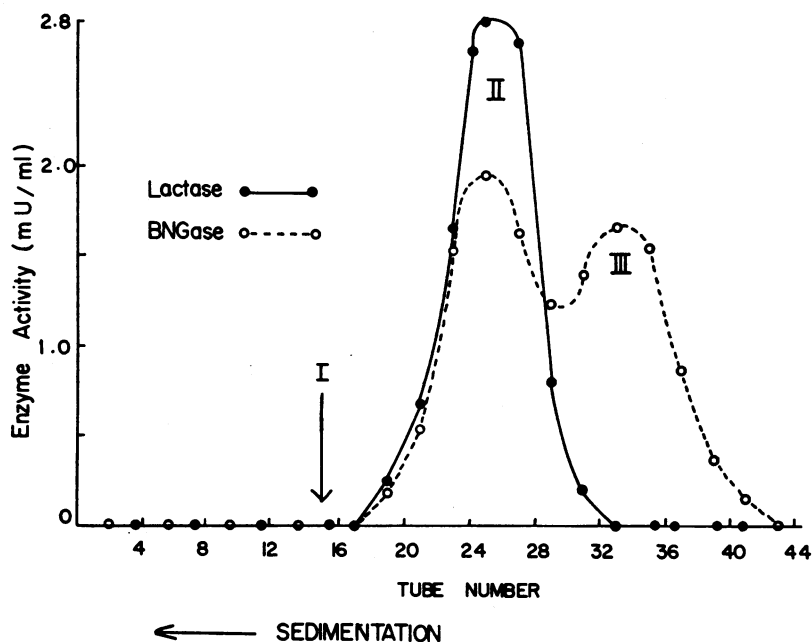


FIGURE 2 Density gradient ultracentrifugation of intestinal preparation of lactase-deficient intestine (patient M.F., Table II) on 5-20% NaCl for 9 hr at $158,000 \times g$. Note the absence of enzyme I that is normally found at fractions 13-15. Enzymes II and III remain at normal levels.

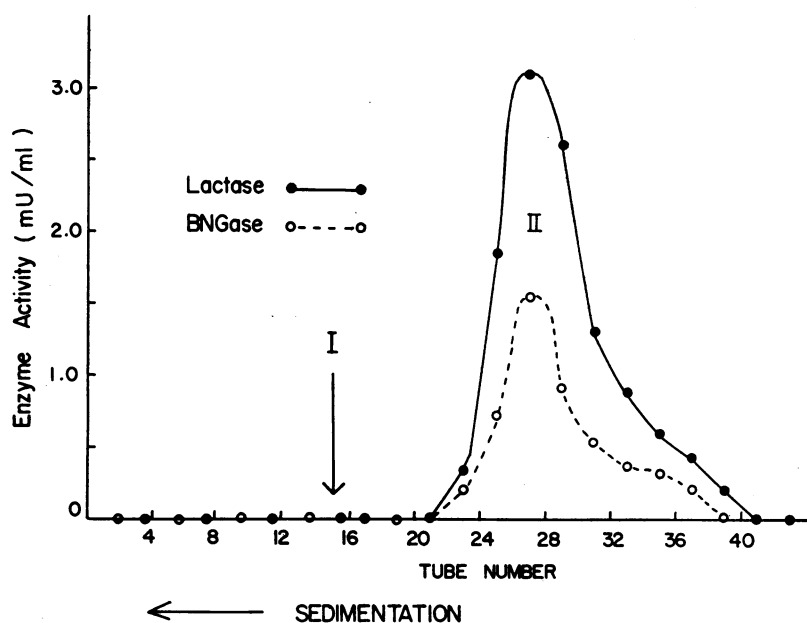


FIGURE 3 Density gradient ultracentrifugation of intestinal preparation from patient P.R. (see Table II) demonstrating not only absence of enzyme I but also of enzyme III, the second BNGase, in fractions 35-37. The slight trailing of both lactase and BNGase probably represents fragments of enzyme II, since fractions 32-38 had biochemical characteristics (pH optimum, specificity, kinetics) typical of enzyme II. Conditions for centrifugation are the same as in Fig. 2.

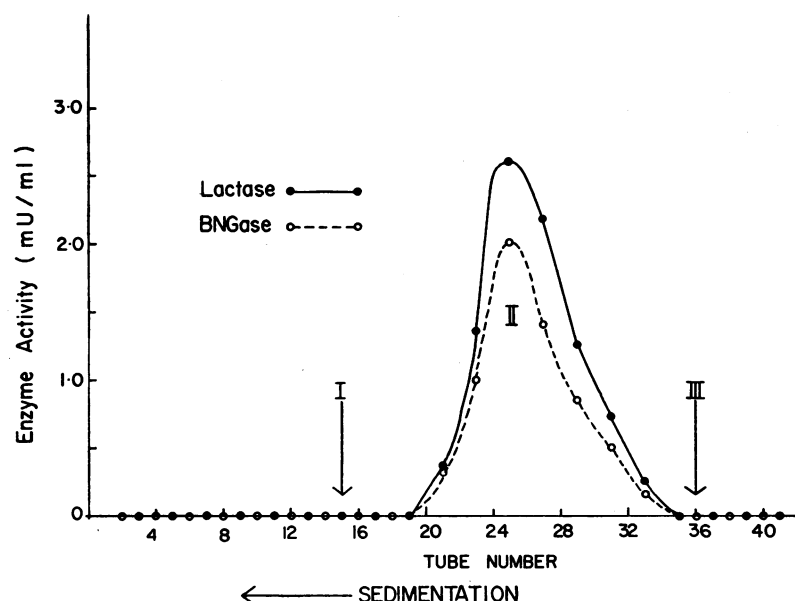


FIGURE 4 Density gradient ultracentrifugation of intestinal preparation from lactase-deficient patient (J. R.) demonstrating absence of enzymes I and III. Enzyme II shows normal activity. Conditions are as in Fig. 2.

RESULTS

pH activity studies of intestinal homogenates. Whole homogenates of biopsies from 10 patients with normal intestinal lactase activity were pooled and compared with those from 10 lactase-deficient patients, as shown in Fig. 1. Whereas normal intestine had a neutral pH activity curve for lactase with a pH optimum of about 5.8, verifying the previous findings of others (16), there was an appreciable shift of the pH activity curve toward the more acid range in lactase deficiency with a pH optimum of 4.8 (Fig. 1).

A summary of similar study of pH activity relationships for individual biopsies is shown in Table I. In

this group of seven adults and six children with lactase deficiency, only one patient had optimal activity at the normal pH of 5.8. All others had the major pH optimal peak at 4.8. Five of the 13 patients had an additional minor peak (less than 50% of the pH 4.8 peak) of activity at pH 6.5. Similar changes in pH optimum were found in tropical sprue, trichioriasis, and idiopathic lactase deficiency, suggesting the biochemical defect may be similar in these varied conditions; furthermore, the consistent shift in pH optimum made it necessary to invoke more than simple depression of all intestinal lactases to account for the alteration in the deficiency state, and hence the individual β -galactosidases were studied as described below.

TABLE II
Clinical Data and Enzymic Defects for Lactase-Deficient Patients

Patient	Racial group	Principal diagnosis	Duration milk intolerance	Intestinal histology	β -Galactosidases missing
P.R.	Puerto Rican Negro	Idiopathic lactase deficiency	20 yr	Normal	I and III
N.A.	American Negro	Cholecystitis	15 yr	Normal	I and III
M.F.	Mexican-American	Intestinal malabsorption ? cause	1 yr	Short villi; Increased lymphocytes	I only
C.K.	Caucasian	Intestinal obstruction Meckel's diverticulum	None	Normal	I only
J.R.	Caucasian	Tropical sprue	1 yr	Short villi	I and III

Density gradient ultracentrifugation. Normal and lactase-deficient intestinal materials were studied individually by density gradient centrifugation. Peroral biopsy material from normal intestine demonstrated the same sedimentation pattern in 5–20% NaCl as had been found for surgical or autopsy material (12). The supernatant preparations proved to be the most useful for study of the enzymes. After 9 hr of centrifugation, peaks of activity for enzymes I, II, and III were in fractions 13–15, 25–27, and 35–37 respectively. 2-hr experiments revealed the macromolecular form of enzyme II in fraction 34.

As outlined in Figs. 2–4, all lactase-deficient tissue showed complete absence of enzyme I, the lactase with pH optimum of 6.0, and molecular weight of 280,000 (12). Three of the five patients also were missing enzyme III, the β -galactosidase with specificity only for artificial substrates (Figs. 3 and 4). All lactase-deficient patients maintained enzyme II at normal levels, and one patient appeared to possess a small molecular weight fragment of enzyme II (Fig. 3). The macromolecular form of enzyme II was also present at normal levels, as estimated by 2-hr gradient centrifugation experiments.

Table II contrasts the different features of the five patients studied by this technique. Two of the three patients with deficiency of both β -galactosidases were Negroes with a long history of milk intolerance not associated with intestinal disease. One white patient with tropic sprue also showed the double-defect. The other two patients, who were missing only enzyme I, were Caucasians with intestinal disease and a short or negative history of milk intolerance. These findings, when taken together with the observations in the preceding paper that enzymes I and III are both peculiar to in-

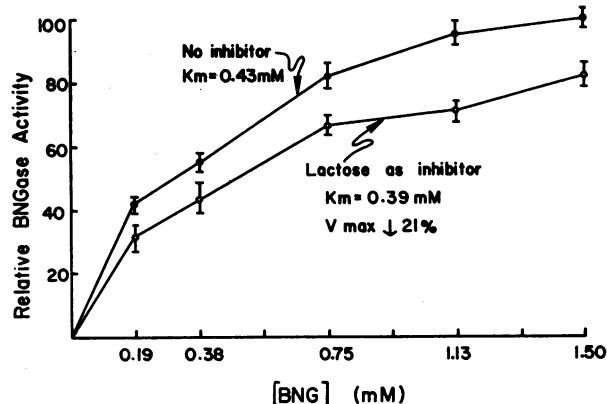


FIGURE 5 Inhibition of enzyme III by lactose. The effect of 70 mM lactose in the reaction mixture on BNGase activity is shown. The predominant change was the depression of the maximal reaction rate (V_{max}). See text for interpretation of kinetics. Mean $\pm$ 2 SE of four experiments.

TABLE III
Comparative Substrate Specificities of Enzymes I and III*

Substrate	Enzyme	
	I	III
β -Galactosides		
Lactose	1.0†	0§
BNG	0§	1.0†
ONPG	0.1	4.7
PNPG	0.2	23
β -Glucosides		
Cellobiose	0.1	0§
BNGlu	0§	1.2
PNPGlu	0.5	13

* BNG = 6-bromo-2-naphthyl- β -galactoside; ONPG = *o*-nitrophenyl- β -galactoside; PNPG = *p*-nitrophenyl- β -galactoside; BNGlu and PNPGlu indicate the corresponding β -glucosides.

† Unity arbitrarily assigned to activity against lactose for enzyme I and against BNG for enzyme III.

§ Less than 0.01 (1%) of reference activity.

testine with identical pH activity curves, prompted further study to determine whether the enzymes are somehow related.

Enzyme III isolated from density gradients: inhibition by lactose. Although enzyme III has specificity only for BNG and other synthetic β -galactosides, the effect of lactose on activity of this enzyme was determined by including lactose at 70 mM in the reaction mixture. As outlined in Fig. 5, definite inhibition of BNGase activity occurred at all substrate concentrations of BNG. Control experiments run with 70 mM sucrose showed no inhibition. When the kinetics for lactose effect were analyzed, the type of inhibition was not typical of either competitive or noncompetitive inhibition, there being some effect on both the Michaelis constant (K_m) and the maximal reaction rate (V_{max}). The most impressive change was a 20% lowering of the reaction rate. The inhibition is best described as being a combination of competitive and noncompetitive types (17).

β -glucosidase activity of enzymes I and III. Since enzyme I is capable of hydrolyzing cellobiose (12), which is a β -glucoside, other β -glucosides were tested as substrates for enzymes I and III. Table III summarizes the relative activities against representative β -galactosides and β -glucosides. Whether β -galactosides or β -glucosides were used as substrates, enzyme I possessed activity against the natural disaccharides but not against the bromo-naphthyl derivatives. In contrast, enzyme III was most active against the synthetic substrates and had no detectable activity against the natural sugars. Notably both enzymes showed appreciable activity against *p*-nitrophenyl- β -glucoside, and each enzyme was

active against an additional β -glucoside. Thus both enzymes are β -glucosidases as well as β -galactosidases. In contrast enzyme II did not possess any β -glucosidase activity.

DISCUSSION

Lactase deficiency, whether secondary to intestinal disease or unrelated and therefore idiopathic or "primary," is associated with a shift in the pH optimum for intestinal lactase in intestinal homogenates from the normal of 5.8 to 4.8. This biochemical alteration is explained by evidence from density gradient centrifugation experiments that a lactase with pH optimum of 6.0 and molecular weight of 280,000 is uniformly undetectable and presumably absent in patients with lactase deficiency. Residual activity is provided by another lactase (pH optimum 4.5, molecular weights 156,000 and 660,000) that remains at normal levels, resulting in the shift to the more acid pH for the whole intestinal homogenate.

The additional peak of activity at pH 6.5 in intestinal homogenates of some lactase-deficient patients is difficult to explain since the enzyme with optimal activity in that pH range is presumably absent. The second peak was always a minor one, being less than 50% of the 4.8 peak. Perhaps the main residual activity has shifted far enough into the acid range so that a small amount of residual lactase activity at higher pH can be easily detected. This would indicate that the deficiency is not complete in some patients, a finding not unexpected if the enzyme depression were secondary to intestinal disease. Thus three of the six patients with tropical sprue demonstrated a second minor peak at pH 6.5 (Table I). Of course it is often impossible to determine whether lactase deficiency is primary and perhaps genetic (1, 2, 18) or secondary because the defect may persist as the sole residual of intestinal disease long after evidence of illness has disappeared (13). We found no evidence for a lactase, either enzyme I or an aberrant enzyme, with pH optimum of 6.0–6.5 in the five lactase-deficient patients whose intestinal biopsies were studied by density gradient centrifugation. It is possible that slight residual activity of enzyme I is below the limits of detection of the density gradient technique. At any rate there seems to be no doubt that enzyme I is either markedly depressed or absent in lactase deficiency.

Elliott and Maxwell (19), utilizing a small Sephadex G-200 column, have reported that a large molecular weight lactase is depressed, whereas a lactase of smaller size persists in Australian aboriginal children with flat oral lactose tolerance tests. Using larger columns, we demonstrated only partial separation of the lactases (12). Therefore, small Sephadex columns can be expected to yield only a single peak containing both lactases. It is likely that the "smaller size" enzyme reported by these

authors (19) actually represents glucose released from the column by action of intestinal α -dextrinase in the sample studied (12) since they apparently interpreted the presence of glucose in the column effluent to be evidence for lactase activity. Unfortunately gel filtration is not suitable for complete separation of the intestinal β -galactosidases. Density gradient ultracentrifugation appears to be a far superior tool for this purpose.

Even though the residual lactase activity provided by enzyme II is 20–70% that of enzyme I, it does not appear to be functional in the digestion of dietary lactose since patients missing enzyme I are intolerant to the ingestion of lactose. This is strong indirect evidence that enzyme II is not located at the luminal pole of the intestinal cell but rather is situated deep within the cell, probably within lysosomes. As outlined in the previous paper (12), its primary substrates may well be gangliosides.

An interesting finding in this study was the deficiency of enzyme III in three of the five patients who had absence of enzyme I (Fig. 4). This enzyme showed no ability to split lactose or cellobiose but was quite active against BNG and the nitrophenyl β -galactosides (12). Despite the obvious differences of these two enzymes, in particular the fourfold difference in molecular weight, they do have several features in common since both are found only in intestine, have pH activity curves that are essentially identical (12), and are capable of hydrolyzing β -glucosides as well as β -galactosides. Furthermore, enzyme III is inhibited by lactose (Fig. 4) but not by another disaccharide, sucrose. Although the inhibition between lactose and BNG was not purely competitive, both V_{max} and K_m were altered, indicating that a mixture of competitive and noncompetitive effects occurred (17). Thus it appears that lactose binds to the enzyme and interferes with interaction of it with its primary substrate, the synthetic β -galactoside. The similarities of enzymes I and III suggest that enzyme III is a fragment or even a precursor of the larger enzyme I, but further studies are required before the exact interrelationship can be defined.

These experiments demonstrating the absence in some patients of both a lactase and an enzyme with specificity only for synthetic substrates assist in explaining the observation of Cook and Dahlqvist (10) that appreciable depression of not only lactase but also of activity against BNG and other synthetic substrates occurs in lactase-deficient Bantu patients. It is reasonable to infer that Bantus are deficient in both enzymes I and III as were some of our patients (Table II).

If enzyme III were a precursor of enzyme I, there could well be two types of biochemical defects leading to enzyme I deficiency and consequent lactose intolerance depending on whether synthesis of precursor or

enzyme I itself were blocked. Of course, the establishment of separate defects characteristic of different types of deficiency will have to await more detailed studies of interaction of enzymes I and III.

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