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NEUTRAL HETERO-β-GALACTOSIDASE FROM RABBIT SMALL INTESTINE

JOHN D. JOHNSON

Department of Pediatrics, Division of Developmental Biology and Nutrition, Stanford University School of Medicine, Stanford, Calif. 94305 (U.S.A.)

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SUMMARY

A hetero-β-galactosidase activity has been identified, characterized and partially purified from the small intestinal mucosa of the rabbit. This enzyme is localized in the cytosol fraction, has a neutral pH optimum, is low molecular weight and has activity towards various synthetic β-galactosides and β-glucosides, but minimal activity towards lactose. Its activity is greater in jejunum than ileum and greater in villus than crypt cells. It is not inhibited by 70–85 mM lactose, but is markedly inhibited by 10^{-4} M *p*-chloromercuribenzoate (PCMB). Colchicine administration results in a parallel depression of the activities of this hetero-β-galactosidase and neutral lactase. The developmental patterns of activity of this hetero-β-galactosidase and neutral lactase diverge significantly. Immunologic studies do not reveal any cross-reaction between cytosol hetero-β-galactosidase and lactase.

Although the hetero-β-galactosidase activity described in this report has many properties in common with similar enzyme activities in the monkey and human, and also with neutral lactase activity, no definite precursor–product relationship between this enzyme and neutral lactase can be established by this study.

INTRODUCTION

Numerous studies have shown that the intestinal mucosa of various mammalian species contains at least two β-galactosidases[1–4]. One of these enzymes is localized in the brush border, has primary specificity for lactose and acts at a neutral pH optimum. This enzyme is responsible for hydrolysis of dietary lactose. The second β-galactosidase is localized in lysosomes and is primarily active against synthetic β-galactosides such as *o*-nitrophenyl-β-galactoside (ONPG) at an acid pH optimum. In 1962, Doell and Kretchmer[1] showed that rabbit intestinal mucosa contains, in addition, a soluble β-galactosidase with minimal activity towards lactose.

Recent studies by Gray and co-workers[5,6] using the human intestine and by

Abbreviations: PCMB, *p*-chloromercuribenzoate; ONPG, *o*-nitrophenyl-β-galactoside; BNG, 6-bromo-2-naphthyl-β-galactoside.

Swaminathan and Radhakrishnan[7] with the monkey intestine have shown that a third β-galactosidase is also present in the high-speed supernatant fraction of homogenates of intestinal mucosa from these two species which does not hydrolyze lactose, but is active toward several synthetic β-galactosides and has a neutral pH optimum. The enzyme activity from human intestine is partially inhibited by lactose. In several patients with lactase deficiency, the neutral lactase and also the neutral hetero-β-galactosidase were both missing. Because of the similarities between the two enzymes, Gray *et al.*[6] have suggested that the neutral hetero-β-galactosidase may be a precursor or fragment of lactase.

This report concerns the characterization and partial purification of a hetero-β-galactosidase from the cytosol of rabbit small intestine. This enzyme has many similarities to the neutral hetero-β-galactosidase of human and monkey small intestine, but no immunologic cross-reactivity with brush border lactase.

MATERIALS AND METHODS

Animals and tissue preparation

New Zealand white rabbits maintained on Purina rabbit chow were used in all these studies. Animals were fasted overnight before use and killed by decapitation. The first third of the small intestine was rapidly removed and chilled in ice-cold 0.9% NaCl. The intestine was rinsed with cold NaCl, slit longitudinally and the mucosa scraped from the muscularis with a steel spatula. The mucosa was homogenized 1:10 w/v in 0.25 M mannitol and a portion of the homogenate centrifuged at 105 000 × *g* for 60 min. The sediment was resuspended in 0.25 M mannitol. Enzyme assays were performed on the whole homogenate, the resuspended sediment and the high-speed supernatant.

Enzyme assays

Lactase activity was determined by a modification of the method of Dahlqvist[8] at pH 5.8 in the presence of 10^{-4} M *p*-chloromercuribenzoate (PCMB). The concentration of lactose in the reaction mixture was 0.15 M. Protein-free filtrates of the reaction mixture were prepared with $Ba(OH)_2$ and $ZnSO_4$; glucose was determined using glucose oxidase (Glucostat Special was purchased from Worthington Biochemical Corporation, Freehold, N.J.) buffered in 0.025 M Tris–HCl, pH 7.0.

Nitrophenyl-β-galactosidase activity was determined with 12 mM *o*-nitrophenyl-β-galactoside (ONPG) at pH 5.5 and 3.5, usually without PCMB, according to Koldovsky *et al.*[9]. Activity using 6-bromo-2-naphthyl-β-galactoside (BNG) as substrate was measured by the method of Dahlqvist *et al.*[10] using 2.0 M Tris rather than 1.0 M to obtain adequate buffering in the diazotization reaction[2]. When *p*-nitrophenyl-β-glucoside was used as substrate, its concentration was 12 mM, and activity was determined at pH 5.5 by the same method as when using ONPG as substrate.

All enzymes incubations were carried out for 10–60 min at 37 °C and were linear with time; activity was proportional to enzyme concentration. A unit of enzyme activity is defined as 1.0 μmole substrate hydrolyzed/h per mg protein. Protein was determined by the method of Lowry *et al.*[11] with bovine serum albumin as the standard.

Enzyme distribution in the functional unit of intestine

The distribution of activities of the β-galactosidases in jejunum was analyzed sequentially from villus to crypt using the technique of Genderen and Engel[12], as modified by Dahlqvist and Nordstrom[13]. The tissue was cut transversely with a cryostat into 80–90 sections approximately 10 μm thick. Each 10th slice was examined histologically and pools of nine consecutive slices analyzed for enzyme activities.

Partial purification of enzymes

Neutral lactase was partially purified from small intestinal homogenate by the following method: Mucosal scrapings were homogenized 1:10 (w/v) in 0.25 M mannitol and centrifuged at 105 000 × *g* for 60 min at 4 °C. The pellet was suspended in 0.25 M mannitol to the original volume of the homogenate. The resuspended pellet was quickly frozen on dry ice, then thawed. This preparation was incubated with 2 mg papain/g wet weight original mucosa and an equal weight of cysteine for 10 min at 37 °C, the reaction stopped by adding PCMB to a concentration of 10^{-4} M and chilling and then centrifuged at 105 000 × *g* for 60 min. Acetone was added to the supernatant to a final concentration of 40% and this mixture was allowed to stand at room temperature for 15 min. After centrifugation, the remaining supernatant was brought to 65% acetone, centrifuged, and the pellet suspended in a minimal amount of 0.01 M sodium citrate, pH 6.0. These steps resulted in a 25-fold purification of lactase. This material was concentrated by vacuum dialysis against 0.01 M sodium citrate, pH 6.0 containing 0.1 M KCl, then run through a Sephadex G-200 column (2.5 cm × 45 cm) equilibrated with the same sodium citrate–KCl buffer solution at a flow rate of 10 ml/h. The fractions containing significant lactase activity (between 65–85 ml) were pooled and vacuum dialyzed against 0.9% NaCl. This material had a specific activity of 110 μmoles/h per mg protein which represents a 500-fold purification with 12% yield.

The ratio of activity at pH 5.5 to that at pH 3.5 was 27:1 and lactase activity was not inhibited by 10^{-4} M PCMB, indicating that it was free of lysosomal acid β-galactosidase[9]. Sephadex gel filtration resulted in complete separation from the soluble neutral hetero-β-galactosidase (see below). This partially purified preparation was utilized for the production of antibody against lactase.

Partial purification of the soluble hetero-β-galactosidase was achieved by gel filtration of the 105 000 × *g* supernatant from small intestinal homogenate on Sephadex G-200. The high-speed supernatant fraction was dialyzed against 0.01 M sodium acetate, pH 6.0, containing 0.1 M KCl, then applied to a 2.5 cm × 45 cm column of Sephadex G-200 equilibrated with the same buffer solution. The flow rate was 12 ml/h. Fractions containing significant *o*-nitrophenyl-β-galactosidase activity (130–175 ml) were pooled and found to have an *o*-nitrophenyl-β-galactosidase/lactase ratio of 1400:1. This minimal activity of lactase was further reduced by chromatography on DEAE-cellulose. The pooled fractions were dialyzed against 0.01 M sodium phosphate, pH 6.0, and chromatographed on a 1.0 cm × 15 cm column of DEAE-cellulose equilibrated with the same buffer. The column was developed with 0.04 M sodium phosphate, pH 6.0, and the majority of *o*-nitrophenyl-β-galactosidase activity was eluted between 15–50 ml. By this procedure, the ratio of *o*-nitrophenyl-β-galactosidase/lactase increased to 5550:1. A second Sephadex G-200 chromatographic step resulted in a final ratio of 13 000:1. The *o*-nitrophenyl-β-galactosidase activity was

101 μmoles/h per mg protein, which represents a purification of 26-fold from the original high-speed supernatant with 17% yield. This preparation was used for antibody production.

Immunologic procedures

Antibodies against lactase were produced by injecting guinea pigs with 100 μg of partially purified lactase combined with 0.5 ml of complete Freund's adjuvant (half subcutaneously, half intraperitoneally) at weekly intervals for 3 weeks. Blood was collected 2 weeks after the last injection and the separated serum stored at −20 °C.

Antibodies against the hetero-β-galactosidase were produced similarly by injecting guinea pigs with 300 μg of partially purified enzyme at weekly intervals and collecting serum 10 days after the third injection. Control serum was collected from the same animals prior to antigen injections. Precipitin reactions were carried out according to the procedure of Feigelson and Greengard[14]. Rabbit anti-guinea pig serum was added to obtain more effective precipitation in some experiments.

RESULTS

Identification and partial purification of neutral hetero-β-galactosidase

Preliminary experiments using adult rabbit jejunum confirmed the finding of Doell and Kretchmer[1] that 75–85% of the total *o*-nitrophenyl-β-galactosidase activity at pH 5.5 of the homogenate was present in the 105 000 × *g* supernatant fraction and that the ratio, lactase (pH 5.8) to *o*-nitrophenyl-β-galactosidase (pH 5.5), was much less in the high-speed supernatant than in the homogenate (Table I). These results suggested that the high-speed supernatant fraction contained a β-galactosidase activity with little or no associated lactase activity. This assumption was confirmed by gel filtration of 105 000 × *g* supernatant of rabbit jejunum on Sephadex G-200. Fig. 1 shows that *p*-nitrophenyl-β-glucosidase (and *o*-nitrophenyl-β-galactosidase) activity in cytosol is distributed into two clearly separable peaks with only the high molecular weight peak demonstrating significant lactase activity (pH 5.8). The major portion of *o*-nitrophenyl-β-galactosidase activity in the cytosol fraction is of low

TABLE I

SUBCELLULAR DISTRIBUTION OF LACTASE AND *o*-NITROPHENYL-β-GALACTOSIDASE ACTIVITIES IN ADULT RABBIT JEJUNAL MUCOSA

Crude subcellular fractions were obtained by differential centrifugation of a 1:10 homogenate in 0.25 M mannitol. The pellet obtained at 300 × *g* for 10 min was designated nuclear, that obtained at 10 000 × *g* for 10 min, mitochondrial, that obtained at 105 000 × *g* for 60 min, microsomal. The pellets were resuspended in 0.25 M mannitol to a volume equal to that of the original homogenate and all fractions were assayed for lactase and *o*-nitrophenyl-β-galactosidase (pH 5.5).

Fraction	*Enzyme activity (units/ml)*		
	Lactase	*o-nitrophenyl-β-galactosidase*	*Lactase/o-nitrophenyl-β-galactosidase*
Homogenate	5.3	39.6	0.13
Nuclear	0.5	3.9	0.13
Mitochondrial	0.6	0.8	0.75
Microsomal	3.0	2.4	1.25
Supernatant	0.9	34.2	0.026

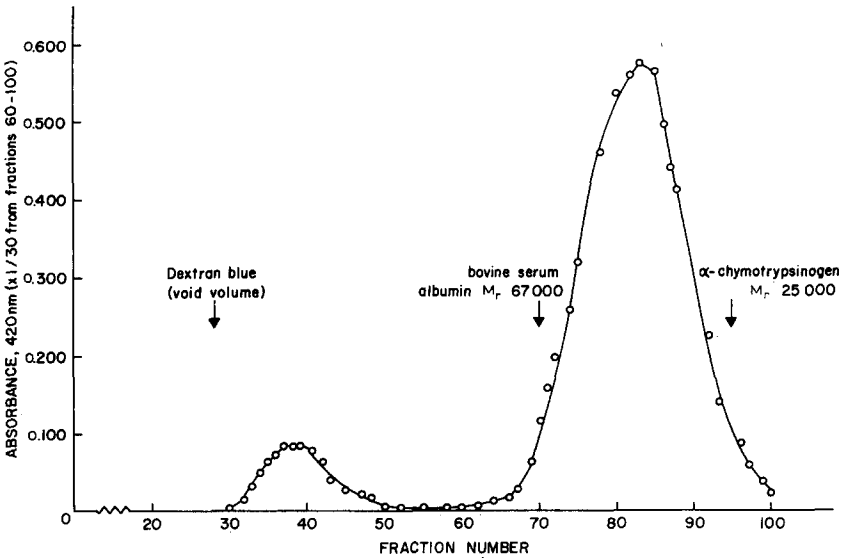

Fig. 1. Sephadex G-200 gel filtration of 105 000 × *g* supernatant. The high-speed supernatant fraction of a 1:10 homogenate of adult rabbit jejunal mucosa was dialyzed overnight against 0.01 M sodium citrate (pH 6.0), 0.1 M KCl, concentrated 2-fold by vacuum dialysis and 1.0 ml (8 mg protein) placed on Sephadex G-200 (45 cm × 2.5 cm) along with 2.5 mg dextran blue, 5 mg α-chymotrypsinogen and 10 mg bovine serum albumin. The column was developed with 0.01 M sodium citrate (pH 6.0) in 0.1 M KCl at a flow rate of 11 ml/h. 2-ml fractions were collected and 150-μl aliquots of each fraction assayed for hetero-β-galactosidase activity using *p*-nitrophenyl-β-glucoside as substrate and a 30-min incubation period. The absorbance values for *p*-nitrophenol in Fractions 60–100 are 30-fold higher than indicated on the scale of the ordinate. The elution pattern of *o*-nitrophenyl-β-galactosidase activity was almost identical to that shown for *p*-nitrophenyl-β-glucosidase. Fractions 35–45 contained a small amount of lactase activity which was not inhibited by 10^{-4} M PCMB and had no activity towards lactose at pH 3.5. Lactase activity was not detected between Fractions 70–90 until 10-fold concentration was achieved.

molecular weight and has little detectable activity towards lactose. After further purification of the low-molecular weight *o*-nitrophenyl-β-galactosidase, the ratio of *o*-nitrophenyl-β-galactosidase (pH 5.5)/lactase (pH 5.8) was increased from 1400:1 to 13 000:1. The properties of this β-galactosidase present in the cytosol of rabbit intestinal mucosa are described below.

TABLE II

SUBSTRATE SPECIFICITY OF CYTOSOL HETERO-β-GALACTOSIDASE

The enzyme preparation used in these experiments was the highly purified fraction used for immunization described in Methods. K_m values were derived from Lineweaver–Burk plots. The substrate concentrations from which the activity ratios were obtained were 12 mM for ONPG and *p*-nitrophenyl-β-glucoside and 0.80 mM for BNG. The *V* for *p*-nitrophenyl-β-glucoside was arbitrarily set at 1.0. All activities were determined at pH 5.5.

Substrate	K_m *(mM)*	*V*
p-Nitrophenyl-β-glucoside	—	1.0
ONPG	2.2	0.5
BNG	0.61	0.1

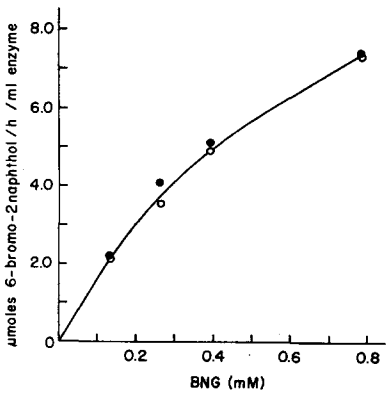

Fig. 2. Effect of lactose on 6-bromo-2-naphthyl-β-galactosidase activity of cytosol hetero-β-galactosidase. Partially purified cytosol hetero-β-galactosidase was incubated with (○) and without (●) 85 mM lactose and varying concentrations of 6-bromo-2-naphthyl-β-galactoside. 6-bromo-2-napthol hydrolysis from *BNG* was determined as described in Methods. Similar results were obtained when ONPG was used as substrate in the presence and absence of 70 mM lactose.

Characteristics of cytosol β-galactosidase

The pH optimum of cytosol β-galactosidase with ONPG as the substrate was found to be 6.0–6.5, which is similar to the pH activity curve for lactase in the 105 000 × *g* sediment. This enzyme is a hetero-β-galactosidase with activity towards ONPG and BNG; it also has β-glucosidase activity with *p*-nitrophenyl-β-glucoside as a substrate (Table II). The presence of 70–85 mM lactose in the reaction mixture did not inhibit the activity of the enzyme toward either ONPG or BNG (Fig. 2).

PCMB (10^{-4} M) completely inhibited partially purified cytosol β-galactosidase (ONPG as substrate), markedly reduced neutral *o*-nitrophenyl-β-galactosidase activity in the unfractionated high-speed supernatant of a jejunal homogenate, but had no significant inhibitory effect on neutral lactase activity in whole homogenate or any subcellular fraction.

TABLE III

DISTRIBUTION OF β-GALACTOSIDASE ACTIVITIES BETWEEN JEJUNUM AND ILEUM OF RABBIT INTESTINE.

Lactase activity was determined at pH 5.8 (with PCMB) and pH 3.5 (without PCMB) on the resuspended 105 000 × *g* sediment of mucosal homogenates from the jejunum and ileum of an adult rabbit. *o*-Nitrophenyl-β-galactosidase activity was determined on the 105 000 × *g* supernatant fractions of the same homogenates.

	Enzyme activity (μmoles substrate hydrolyzed/h per mg protein)	
	Jejunum	*Ileum*
Microsomal lactase, pH 5.8	0.53	0.09
Microsomal lactase, pH 3.5	0.36	0.51
Cytosol *o*-nitrophenyl-β-galactosidase, pH 5.5	6.7	1.9

Distribution of cytosol β-galactosidase in the small intestine

The distribution of β-galactosidase activities between jejunum and ileum was found to be similar for cytosol *o*-nitrophenyl-β-galactosidase activity and neutral lactase activity in homogenate or 105 000 × *g* sediment (Table III), *viz.*, 3–5-fold higher in jejunum than ileum. In contrast, acid lactase (pH 3.5) activity was higher in ileum than jejunum.

An analysis of jejunal β-galactosidase activities determined sequentially from villus to crypt using cryostat sectioning is shown in Fig. 3. Homogenate *o*-nitro-

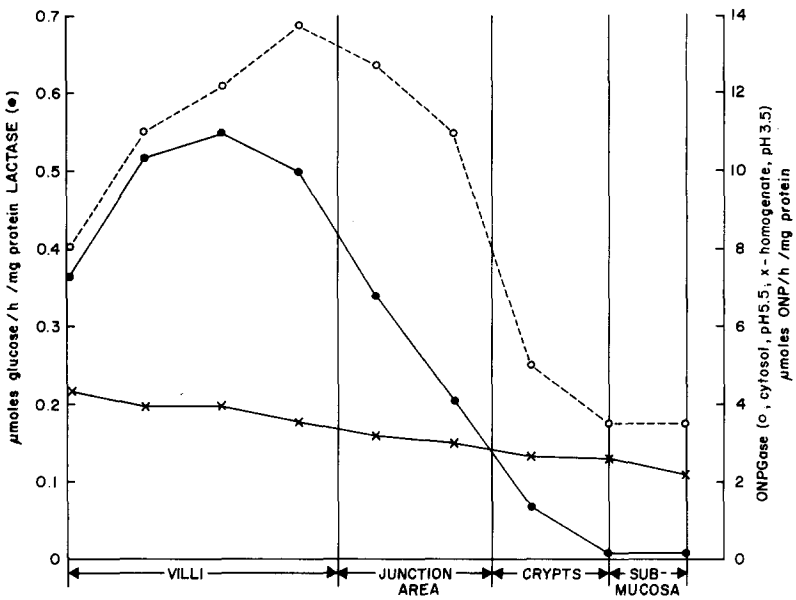

Fig. 3. Vertical distribution of β-galactosidase activities in rabbit jejunum. Cryostat sectioning and enzyme assays were performed as described in Methods. Homogenate lactase, pH 5.8 (●), homogenate *o*-nitrophenyl-β-galactosidase (ONPGase), pH 3.5 (×) and cytosol *o*-nitrophenyl-β-galactosidase (ONPGase), pH 5.5 (○) activities were determined on pools of 9 cryostat sections and each 10th section was examined microscopically.

phenyl-β-galactosidase activity at pH 3.5 is low in all functional units of the mucosa with little change in specific activity from crypt to villus. Neutral lactase activity increases markedly from crypt to villus, following a pattern previously described by Fortin-Magana *et al.*[15] in the rat. Cytosol neutral *o*-nitrophenyl-β-galactosidase also reaches peak activity in the villus cells. The distribution of cytosol β-galactosidase within the intestinal mucosa corresponds closely to that of neutral lactase.

Activity of cytosol β-galactosidase during maturation

Activities of cytosol *o*-nitrophenyl-β-galactosidase (pH 5.5) and lactase in homogenate (pH 5.8, with PCMB) are shown as a function of postnatal age in Fig. 4. Neutral lactase activity is high during the first days after birth, then falls to quite low values in the adult. These results correspond closely with those previously reported for the rabbit by Doell and Kretchmer[1]. The activity of hetero-β-galactosidase in the

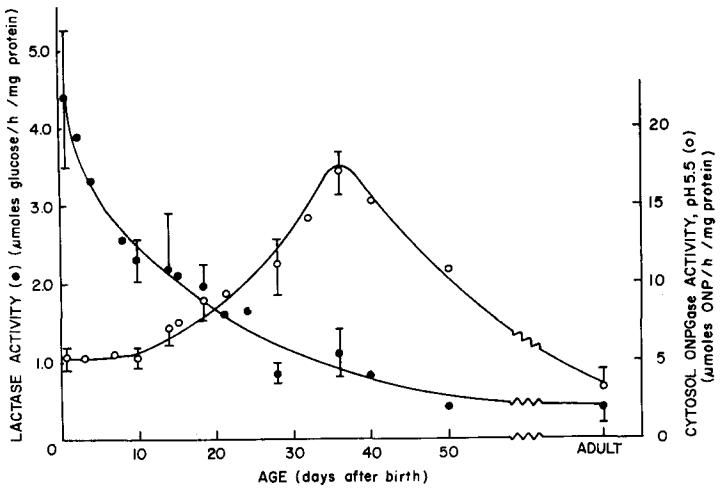

Fig. 4. Developmental curves of activity of lactase and cytosol hetero-β-galactosidase in rabbit jejunum. The values shown represent the mean ±S.E. of from 4–6 animals or the mean of 2 animals when no S.E. is shown.

cytosol fraction follows a complex developmental curve, remaining relatively unchanged during the first 10 days, then increasing to a maximum between 30–40 days, and returning to low activity levels in the adult.

Response to colchicine administration

The subcutaneous injection of colchicine (2 mg/kg) resulted in a parallel decrease in neutral lactase and cytosol β-galactosidase activities at 18–33 h following administration. This effect was not yet apparent 8–10 h after injection, and normal activity was recovered by 42–48 h following colchicine administration. The results of a

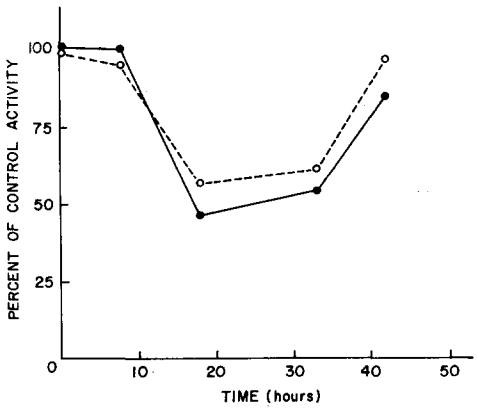

Fig. 5. Effect of colchicine on lactase and cytosol hetero-β-galactosidase activities in rabbit jejunal mucosa. 33-day-old rabbits were injected with colchicine (2 mg/kg, subcutaneously) or 0.9% NaCl (controls), sacrificed at various time points and intestinal mucosa assayed for lactase, pH 5.8 (●) and cytosol *o*-nitrophenyl-β-galactosidase, pH 5.5 (○). The values shown represent the means of 2–4 animals expressed as percent of control activity.

TABLE IV

PRECIPITIN REACTION OF LACTASE WITH ANTI-CYTOSOL HETERO-β-GALACTOSIDASE ANTISERUM

The partially purified enzymes and antiserum or control serum, all dialyzed against 0.9% NaCl, were incubated at 23 °C for 60 min, then kept at 4 °C for 60 h. All tubes, each containing 0.3 ml, were centrifuged at 105 000 × *g* for 60 minutes and the supernatant fractions assayed as described in Methods.

Tube	*Enzyme added (units/tube)*		*Anti-cytosol o-Nitrophenyl-β-galactosidase (ml)*	*Supernatant enzyme (units/tube)*		
	*Lactase**	*Cytosol o-nitrophenyl-β-galactosidase*		*Lactase*	*o-Nitrophenyl-β-galactosidase*	*Δ*
1	0.40	—	0.2	0.40	—	
2	0.40	—	—**	0.41	—	
3	—	2.8	0.02	—	1.1	
						1.7
4	—	2.8	—	—	2.8	
5	0.70	2.8	0.02	—	1.9	
						1.5
6	0.70	2.8	—	—	3.4	

* Lactase activity refers to the activity of the partially purified enzyme using ONPG as substrate.

** A volume of control serum, equivalent to the volume of antiserum in the matched tube, was added to tubes not containing antiserum.

representative experiment are shown in Fig. 5. Sucrase activity also decreased following colchicine injection, but the percent decrease was less than that of the β-galactosidases.

Immunological studies of the β-galactosidases

Both of the partially purified enzymes served as good antigens for the production of precipitating antibodies. Neutral lactase activity was not precipitated by anti-*o*-nitrophenyl-β-galactosidase antibody (Table IV, 1–2), nor did lactase inhibit precipitation of cytosol *o*-nitrophenyl-β-galactosidase by its specific antibody when

TABLE V

PRECIPITIN REACTION OF CYTOSOL HETERO-β-GALACTOSIDASE WITH ANTI-LACTASE ANTISERUM

Precipitin reactions were performed as described in Table IV except that rabbit anti-guinea pig serum was added to each tube after 16 h incubation at 4 °C, tubes allowed to sit at 23 °C for 45 min, then further incubated at 4 °C for 72 h before centrifugation.

Tube	*Enzyme added (units/tube)*		*Anti-lactase (ml)*	*Supernatant enzyme (units/tube)*	
	Cytosol o-nitrophenyl-β-galactosidase	*Lactase*		*o-Nitrophenyl-β-galactosidase*	*Lactase*
1	0.69	—	0.1	0.69	—
2	0.69	—	—	0.80	—
3	—	0.54	0.01	—	0
4	—	0.54	—	—	0.65
5	0.69	0.54	0.01	—	0
6	0.69	0.54	—	—	0.53

both enzymes were present (Table IV, 3–6). Anti-lactase antibody did not precipitate cytosol *o*-nitrophenyl-β-galactosidase activity (Table V, 1–2), nor did cytosol *o*-nitrophenyl-β-galactosidase inhibit the precipitation of lactase by anti-lactase antibody (Table V, 3–6).

DISCUSSION

Studies of the neutral hetero-β-galactosidase from the cytosol of human small intestine have suggested the possibility that this enzyme is a precursor or subunit of brush border lactase[5,6]. This work was undertaken in an attempt to identify and characterize a similar hetero-β-galactosidase in an animal model with the hope of partially elucidating the cellular mechanisms of lactase synthesis and the biochemical basis for the lactase-deficient state of most species of adult mammals[16].

The partially purified neutral hetero-β-galactosidase from the small intestine of the rabbit described in this report has many similarities to the monkey and human enzymes[5–7]. It is localized in the high-speed supernatant fraction of mucosal homogenate, has a neutral pH optimum, has activity towards synthetic β-galactosides and β-glucosides, but no significant activity towards lactose, and is a low-molecular weight enzyme.

This hetero-β-galactosidase in rabbit intestine also has many properties in common with neutral lactase. It has a neutral pH optimum, is higher in activity in jejunal mucosa than ileal mucosa, and is higher in activity in the villus than in the crypt cells. It is a β-glucosidase as well as a β-galactosidase, and is decreased in activity by colchicine administration in parallel with neutral lactase.

Other findings in this study suggest that these parallelisms should not be regarded as evidence that the neutral hetero-β-galctosidase and lactase from rabbit small intestine are related in a precursor–product fashion. The developmental curves of these two enzymes differ significantly (Fig. 4). Lactose does not inhibit cytosol hetero-β-galactosidase activity toward ONPG or BNG as is the case with the human enzyme. PCMB inhibits cytosol hetero-β-galactosidase but not lactase. Immunological studies show no cross-precipitation of lactase by antibody against the hetero-β-galactosidase; similarly, there is no precipitation of hetero-β-galactosidase activity by anti-lactase antibody. Neither enzyme inhibits precipitation of the other by their specific precipitating antibodies. With the use of these techniques, there is no immunological cross-reaction between the two enzymes. Although the parallel depression in lactase and cytosol hetero-β-galactosidase activities following colchicine administration supports an interrelationship between the two enzymes, recent studies by Herbst *et al.*[17,18] show decreased activities of several intestinal mucosal enzymes in addition to lactase following colchicine administration.

Similar cytosol hetero-β-galactosidase activity is not found in all mammalian species. We have not been able to identify such an enzyme activity in the cytosol of jejunal mucosa from the rat. Dahlqvist *et al.*[19] described an enzyme from rat intestinal mucosa with 6-bromo-2-naphthyl-β-galactosidase activity but no lactase activity following TEAE-chromatography of papain-treated homogenates. We have found a similar enzyme activity in the intestinal mucosa of the rat after papain treatment of the high-speed sediment, but not in concentrated high-speed supernatant fractions of the homogenate. The enzyme fraction detected after papain treatment of rat intestine

inhibits precipitation of rat lactase by anti-lactase antibody, and is most likely a fragment of lactase produced by proteolysis, whereas cytosol hetero-β-galactosidase from the rabbit small intestine occurs in high activity in untreated high-speed supernatant and is thought not to represent an artifactual enzyme produced by proteolysis.

The physiological function of the cytosol hetero-β-galactosidase from the small intestine of the rabbit remains an enigma. Further studies to delineate a possible relationship between this enzyme and neutral lactase in the rabbit should include a kinetic analysis of the rates of synthesis of the two enzymes at various developmental ages and further immunologic studies of cross-reaction such as the subcellular localization of fluorescent anti-cytosol hetero-β-galactosidase antibody.

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