Kinetic Constants of Lactase of the Small Intestine of Growing Rats Fully Recovered from Protein Depletion, in Relation to Dietary Protein and Lactose Concentration

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Summary The effect of dietary composition on the kinetic constants of intestinal lactase was studied using rats depleted of protein by feeding protein-free diet from the weanling stage to 34 days of age and subsequently allowed to recover on diets containing 11.5 or 17% of protein calories (P%) and different levels of lactose (L%; 1, 15, 27 and 37%). After four days of refeeding, rats were decapitated and lactase activity was determined at different substrate concentrations by the method of Dahlqvist using homogenate of intestinal mucosa. Maximum velocity ($V_{max}$) and Michaelis constant ($K_m$) were calculated according to Eisenthal and Cornish-Bowden. At both levels of P%, $V_{max}$ tended to increase with the dietary lactose concentration. With diets containing 37% lactose, at P% 11 $V_{max}$ was about 60% of that at P% 17.0. $K_m$ tended to increase with L% in groups given the 17.0% protein calorie diets, but no difference was observed between groups fed at the lower level of protein. These results can be explained on the basis of interactions between dietary protein at different concentrations and inducer substrate which results in changes in isoenzyme patterns.

Key Words lactase, small intestine, brush border membrane, growing rats, refeeding

The effects of dietary composition on activities of hydrolytic enzymes bound to the brush border of the small intestine have been studied by several workers, who suggest that digestive enzymes are specifically induced by the respective substrates present in diet. Deren et al. (1) demonstrated a twofold increase in sucrase and

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2 Career investigator CONICET, Argentina.
3 Ana Lia Felipoff and Maria Esther Rio. 66th Meeting of FASEB April, 15–23, 1982.
maltase activities after feeding fasted rats on diets with a high carbohydrate content. Saito and Suda (2), who studied dietary effects on kinetic constants of leucine-aminopeptidase bound to the intestinal brush border in normal adult rats, found higher maximum velocities ($V_{\text{max}}$) when rats were fed on high-casein diets than when given low-casein ones. On the other hand, there are no reports extent on the effect of dietary composition on digestive enzymes of growing rats recovering from protein depletion nor on the interaction between dietary protein at different concentrations and inducer substrate.

In order to provide information on these points, young protein-depleted rats were fed on experimental diets containing lactose and protein at different concentrations. At the end of the refeeding period, kinetic constants of intestinal lactase were studied.

**EXPERIMENTAL**

**Animals.** Early undernourished rats of the Wistar strain were used in this experiment. In order to ensure lactation as uniform as possible, during the suckling period litter size was adjusted to eight. At the weanling stage (21 days of age), groups of 4 rats each were housed in suspended mesh cages. Acute undernutrition was produced by feeding rats from 21 to 34 days of age with a protein-free diet containing 1% lactose. At 34 days of age, weight loss averaged $20.7 \pm 0.6\%$ ($\bar{X} \pm SD$) of the initial body weight. Rats were then separated at random into 8 groups of 8 rats each and were allowed to recover over a period of four days on one of the experimental diets containing different levels of protein and lactose. Room temperature and light were controlled ($21 \pm 1.0^\circ C; 12\text{hr light-dark cycle}$).

**Diets.** The composition of the experimental diets is summarized in Table 1. The

| Ingredient | P%*: 0 | P%: 11.5 | P%: 17.0 |
|------------|--------|----------|----------|
|            | PFD\(b\) | A1 | A15 | A27 | A37 | B1 | B15 | B27 | B37 |
| Casein     | 0.0 | 13.0 | 13.0 | 13.0 | 14.0 | 20.0 | 19.3 | 18.1 | 17.6 |
| Lipids     | 4.5 | 13.0 | 13.0 | 13.0 | 24.0 | 20.0 | 16.5 | 13.3 | 5.0  |
| Minerals and vitamins\(c\) | 6.3 | 6.5 | 6.5 | 6.5 | 7.0 | 6.0 | 8.9 | 7.9 | 10.4 |
| Dextrin c.s.p. | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Lactose    | 1.0 | 0.0  | 15.0 | 27.0 | 37.0 | 0.0  | 14.0 | 27.0 | 37.0 |
| Energy density\(d\) | 3.8 | 4.4 | 3.9 | 4.4 | 4.9 | 4.7 | 4.5 | 4.4 | 3.8  |
| P%         | 0.0 | 11.9 | 11.9 | 11.9 | 11.4 | 17.0 | 17.3 | 16.6 | 18.4 |

\(a\) P%, dietary protein calories concentration. \(b\) PFD, protein-free diet. \(c\) Harper, A.E. (1959): *J. Nutr.*, 68: 405-418. \(d\) Energy density: kcal/g.

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diets provided 11.5% (Diets A) or 17% (Diets B) protein calories, and 4 levels of lactose (L%: 1, 15, 27 and 37%), and supplied adequate amounts of lipids, vitamins and minerals (3). Diets and water were offered ad libitum.

Methods. At the end of the recovery period, diets were withdrawn, and after 4 hr. Fasting rats were decapitated between 12:00 and 14:00 hr (2); the first 40 cm of the small intestine was rapidly removed, washed out with cold 0.9% NaCl and everted. The mucosa was collected by scraping the intestine with a glass slide and homogenized with 2 ml of cold 0.9% NaCl in a Teflon-pestle homogenizer. All procedures were performed at 0–4°C.

Assay procedure. Lactase activities were determined in homogenates of the small intestinal mucosa by the Tris glucose oxidase method of Dahlqvist (4) at different lactose concentrations: 7, 14, 28, 56 and 84 mM in 0.1 M sodium maleate buffer, pH 6.0.

Maximum velocity (Vmax) and Michaelis-Menten constant (Km) were calculated according to the direct linear plot of Eisenthal and Cornish-Bowden (5). Protein was estimated by the method of Lowry et al. (6).

Statistical analysis. Results were analyzed by a variance test according to Scheffé (7) at a significance level of 1%.

RESULTS

At the end of the refeeding period, irrespective of P%, animals recovered their initial body weight, except for those receiving dietary lactose at a concentration of 37%, which only recovered to a level of 94%.

Fig. 1. Effect of experimental diets on the Michaelis-Menten constant of the intestinal lactase of growing rats fully recovered from protein depletion.

* L%, dietary lactose concentration; b P%, dietary protein calories concentration. c Mean ± SD, n=8. * p<0.01.
Fig. 2. Effect of experimental diets on maximum velocity of the intestinal lactase of growing rats fully recovered from protein depletion.

$^a$L%$^b$, dietary lactose concentration; $^b$P%, dietary protein calories concentration. $^c$Mean±SD, n=8. $^*p<0.01$.

**Michaelis-Menten constant**

$K_m$ tends to increase with increased dietary lactose concentration in groups fed on 17.0% protein calorie diets (groups B) (Fig. 1). Conversely, there was no significant influence of dietary lactose concentration on the $K_m$ of the groups refed on diets providing 11.5% of protein calories (groups A). Moreover the $K_m$ for the lowest level of lactose in group B was significantly lower than that in group A.

**Maximum velocity**

$V_{max}$ tends to increase along with dietary lactose concentration when P% is 17.0%, the difference being significant between 1 and 37% ($p<0.01$).

For a dietary protein concentration of 11.5%, the same trend was observed between 15 and 37%, but the difference did not attain the expected level of significance. Anomalous behavior was observed for the lowest level of lactose where the $V_{max}$ surpassed the $V_{max}$ for all the other lactose levels.$^3$ However, due to the large values for the standard error of this group, differences were not significant at the level of $p<0.01$.

When diets provided 37% of lactose, the maximum velocity at P% 11 was about 60% of that at P% 17 (Fig. 2).

**DISCUSSION**

The results of this work confirm for lactase that the $V_{max}$ of enzymes of the
intestinal brush border increase with the level of inducer substrate in the diet. However, Saito and Suda (2) working with adult rats, found interrelations between maltase activity and protein concentration for levels of dietary protein above 75%. Our findings show that in the case of intestinal lactase, growing rats recovering from protein depletion are far more sensitive to changes in dietary protein concentration than in the previous case.

It is interesting to point out that a small change in protein concentration from 11.5 to 17.0 was able to increase significantly (63%) the $V_{\text{max}}$ of lactase as a response to the highest level of lactose in the diet (Fig. 2). Since an influence of P% was not found with lower levels of lactose, this increase seems to result from some adaptive process that would take place at this higher level of inducer substrate. Other differences, with regard to Saito and Suda, were found to lie in the changes observed in $K_m$, whereas no changes were found for those authors, in the present paper significant differences were demonstrated as a consequence of changes in dietary composition related to both protein and lactose concentration. Differences in $K_m$ seem to be more related to protein than to lactose; groups refed on diets A (P% 11.5) did not show any changes as a result of increasing lactose, whereas a close interrelationship was found between $K_m$ and lactose dietary concentration in the groups fed with diets B (P% 17.0). In these last groups a trend of increase of $K_m$ with lactose concentration was observed, so that the difference between groups with 1 and 37% lactose was significant.

On the basis of the results reported here we suggest that the changes in $K_m$ and $V_{\text{max}}$ of intestinal lactase might arise from interactions between:

a) Dietary lactose as the inducer substrate, and,

b) Dietary protein concentration regulating amino acid availability for “de novo” enzymatic synthesis during the recovery period.

Since there are at least 3 different lactase isoenzymes known, changes in the Michaelis-Menten constant would suggest that these interactions would result in changes in isoenzyme patterns. A similar explanation was proposed by Yoshizawa et al. (8) who found changes in $K_m$ and $V_{\text{max}}$ of chick embryonic intestinal maltase as a result of changes in the inducer sugars present in an organ culture system.

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