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A PROPOSED MECHANISM OF NORMAL INTESTINAL LACTASE DECLINE IN THE POSTWEANED MAMMAL

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Summary--Intestinal lactase declines to low levels in the postweaned mammal as an adaptation to normal termination of lactose ingestion. Although a well known phenomenon and presumably the basis of milk intolerance among most human adults, the mechanism of this decline has remained obscure. Evidence is presented to support a proposal that temporally related cytokinetic changes, which effect a generally corresponding reduction in enterocyte life-span, serve as the causal basis of lactase decline in the postweaned mammal.

Ontogenic development continues in the intestine of the infant mammal in adaptation to extrauterine life. Particularly significant redifferentiative changes in epithelial structure, function (1-4) and cytokinetic properties (5,6) occur abruptly over a short critical period around the normal time of weaning. Functional adaptations preparatory to dietary carbohydrate change with weaning are especially remarkable and in the rat include coincident marked decrease in lactase, appearance of active sucrase-isomaltase and rapid increase in maltase activities (3,4,7). Cytokinetic changes also occurring at this time include increased cell proliferation and migration rates, resulting in an abrupt reduction in the enterocyte life-span from an infant pattern of 7-10 days to an adult pattern of 2-3 days when measured in the rat (5,6,8).

A decline in enteric lactase activity occurs as an evolutionary adaptation to the normal termination of lactose ingestion with weaning in all mammals studied containing the enzyme (lactase absent in pinnipeds (9)). Recognition of lactase decline as a natural phenomenon of the postweaned mammal has provided a basis of understanding and study of lactose intolerance exhibited by most of the adult human population (10). Although a phenomenon of biological, anthropological, nutritional and clinical significance, the mechanism of lactase decline has remained obscure. A mechanism involving a gene-regulated suppression of its rate of synthesis appears to be a widely held view (10,11), although without experimental basis.

We present evidence in this report supportive of a proposal that temporally related cytokinetic changes leading to correlative decreases in en-

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terocyte life span provide the primary causal basis of enteric lactase decline in the postweaned mammal. Additional evidence consistent with this proposal has been included in other studies (12,13).

EXPERIMENTAL

Materials - Infant rats were of Wistar strain from our breeding colony. [Methyl-^{3}H] thymidine at 40 Ci/mmole (Amersham), lactose and cellobiose (Sigma Chem. Co.) and Statzyme glucose (Worthington) were purchased from the indicated sources. Rat intestinal lactase was purified to near homogeneity at specific activity of 23 (14).

Lactase Measurements - Rats were sacrificed at 9-10 a.m. and lactase activity was determined on jejunal homogenates of similar areas of intestine or sonicates of isolated intestinal cells. Assays were performed using either 0.15M lactose or 0.015M cellobiose as substrates by procedures described previously (14). Cellobiose shows greater specificity than lactose for the membrane-bound enzyme in the presence of acid β-galactosidase and is degraded at 4.5 times lesser rate under the assay conditions described (14). Lactase activity units are expressed in μmols substrate hydrolyzed per minute and specific activity in activity units per mg protein. Protein was determined by the method of Lowry et al (15).

Cell Fractionations - Intestinal epithelial cells were isolated in sequential fractions from villus tip to inner crypt by adaptation of published procedures (16,17). Everted intestinal segments were gently shaken in Dulbecco's phosphate buffered saline minus cations plus 1.5 mM EDTA and 0.5 mM dithiothreitol and successively liberated cells collected in a number of fractions. The amount of cells present in each fraction was estimated by protein (15) or DNA (18) measurements. The fractionation procedure has been applied in a previous study (13).

Determination of Cell Migration Rates - Rats were injected i.p. with 25 μCi [^{3}H] thymidine at 9 a.m. to label newly formed intestinal crypt cells and sacrificed after specified times. Jejunal segments were removed from similar areas of the intestine and sequential cell fractions isolated from villus tip to lower crypt. Distribution of radioactivity in the cell fractions was determined on sonicates dissolved in 0.1N NaOH with Amersham Aqueous Counting Scintillant in a Beckman LS-223 beta counter. Cell migration rates were determined from the distribution curves of radioactivity.

Histological Measurements - Villus height, crypt depth and mitotic index were determined on tissues fixed in 10% formalin, embedded in paraffin, sectioned along their longitudinal axis and stained with hemotoxylin-eosin.

RESULTS

Lactase Activity Decline - In Fig. 1 are shown lactase activities of comparable jejunal areas from rat pups ranging from 10-22 days of age, using cellobiose as a specific enzyme substrate. The age range was selected to delineate a short critical period of particularly rapid lactase activity decline, occurring over a few days at around the normal weaning age of the rat. Following this decline, lactase activity is maintained at low levels through adult life.

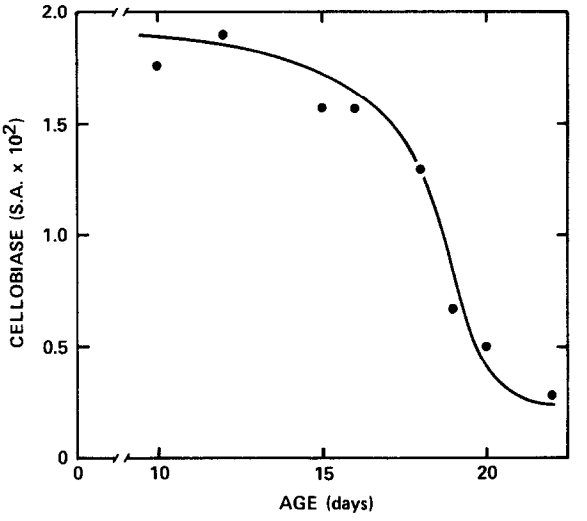

Figure 1. Pattern of lactase activity decline accompanying maturational transition of rat enteric epithelium from pre- to postweaned state. Cellobiose was utilized as a selective substrate for the membrane-bound lactase.

Cytokinetic Relationships - Transition of enteric epithelium from suckling to mature state is accompanied by a shift in the cytokinetic properties of the tissue (5,6). The shift includes an acceleration of cell proliferation and migration rate along the villus column, resulting in a corresponding increase in the turnover rate characteristic of mature enteric epithelium.

Cell migration rates were determined in the present study by the method illustrated in Fig. 2. Distribution patterns of radioactivity in cell fractions from villus tip to inner crypt at 24, 72 and 120 hours following a single injection of [^{3}H] thymidine to 10 day old infant rats are shown. The average migration distances along the villus column of labeled cells originating in crypts after each of the time periods are shown as points on the distribution curves where the relative specific activity is equal to 0.5. A generally linear cell migration rate is seen with time (Fig. 2B), as found in our earlier studies using radioautographic methods (5,6), with the x-intercept of the plotted straight line indicating an average transit time to villus tip of 7 days. The transit time is assumed to approximate the lifespan of the cell.

The migration rates of intestinal cells were determined on rats ranging from 16-21 days of age. The age range was selected to extend through the transitional period of rapid intestinal lactase decline, occurring at about the time of normal weaning. In Fig. 3 are plotted the average migration dis-

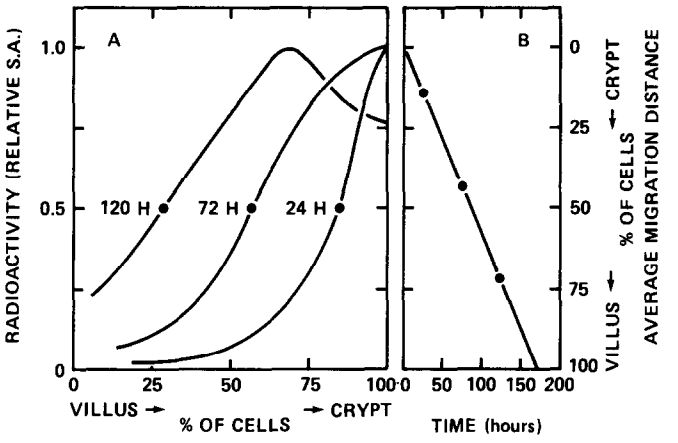

Figure 2. Determination of cell migration rate along the crypt-villus column.

A. Distribution of radioactivity in intestinal cell fractions determined at 24, 72 and 120 hours following [^{3}H] thymidine administration to 10 day old rats. The average cell migration distances are shown as mid-points of the distribution curves.

B. Data replot showing a generally constant cell migration rate and an extrapolated transit time to villus tip of 7 days.

tances in 24 hours of labeled cells along the crypt to villus column in comparable segments of proximal intestine from the selected age group of rats. Cell migration rates are maintained at the slower infant rate through day 17 and accelerate to the faster adult rate over the following few days, achieving

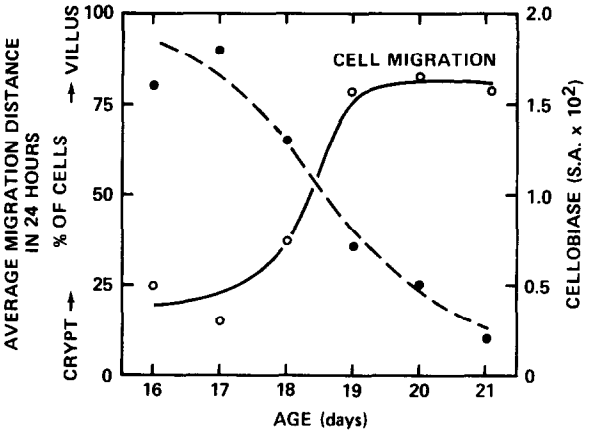

Figure 3. Pattern of changing cell migration rates accompanying transition of rat enteric epithelium from pre- to postweaned state. The average migration distances of labeled cells were determined 24 hours after [^{3}H] thymidine administration. Pattern of lactase decline is included for comparative purposes.

TABLE I. Villus height, crypt depth and mitotic cells of rat intestine determined over the period of cytokinetic transition from infantile to adult cell turnover patterns

Rats[a]	Cell Migration[b] (%)	Villus Height[c] (mm)	Crypt Depth[c] (mm)	Mitotic Cells[d] (No.)
Pre-transition (n = 4)	11 ± 5	0.32 ± 0.01	0.055 ± 0.004	1.1 ± 0.1
Mid-transition (n = 4)	43 ± 9	0.32 ± 0.02	0.063 ± 0.005	1.1 ± 0.2
Post-transition (n = 4)	87 ± 6	0.31 ± 0.03	0.100 ± 0.006	2.0 ± 0.4

[a] Small segments of mid-small intestine from closely related age groups of rats showing pre-, mid- and post-cytokinetic transition were selected for the morphometric measurements.

[b] The average migration distance of labeled cells (expressed as % of distance to villus tip), determined 24 hours following [^{3}H] thymidine administration.

[c] Measurements were performed on the 10 largest crypt-villi units and the average value recorded.

[d] Number of mitotic cells counted per 5 crypt units.

an apparent 3 to 4-fold increase in their migration and presumably turnover rates during this period. The accompanying pattern of declining intestinal lactase activity occurring during this period has been included in Fig. 3 for comparative purposes. A strong correlative relationship is evident between the patterns of lactase activity decline and acceleration of cell migration rate, in both their timing as well as magnitude. We have proposed on this basis that the enterocyte lifespan may be the primary determinant of intestinal lactase activity decline accompanying weaning.

Cytological Relationships - Cytological changes found to accompany cytokinetic transition of enteric epithelium from the infant to adult state are shown in Table I. Intestines from rats of closely related age showing pre-, mid-, and post-transition from infant to adult cell migration rates were selected for the morphometric analyses. The intestines show some increase in crypt depth, greater frequency of mitotic cells and little change in villus height, accompanying transitional change in cell migration rates to the accelerated adult pattern. In the absence of accompanying changes in villus height, increases in cell migration rate should result in a proportional decrease in transit time to the villus tip. Determination of changes in cell migration rate, over this maturational interval, have been considered on this basis to provide a direct measure of resultant changes in the lifespan of the enterocyte.

Lactase Accumulation and Enterocyte Aging - The differentiated enterocyte (columnar epithelial cell) on emerging from the proliferating crypt zone

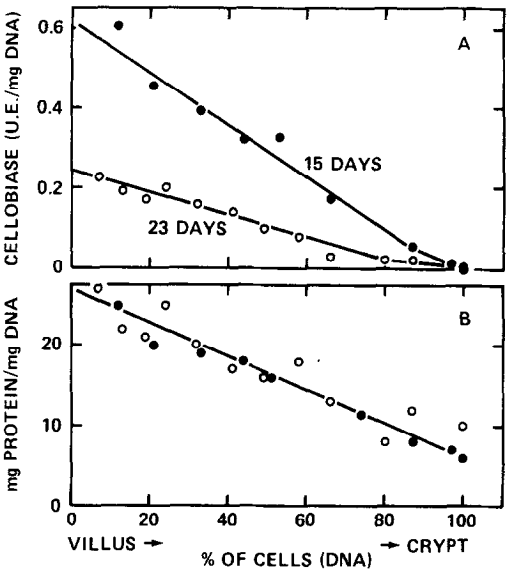

Figure 4. Lactase activity and total cell protein distribution patterns across the crypt-villus cell gradient in pre- and postweaned intestine.

A. Linear increases in lactase activity in proportion to the relative age of the enterocyte.

B. Coincident linear accumulation patterns of total cell protein in pre- and postweaned intestine.

continues to synthesize and accumulate lactase while migrating the length of the villus. In Fig. 4A are shown distribution patterns of lactase activity in intestinal cell fractions isolated sequentially from villus tip to inner crypt from a preweaned 15 day and a postweaned 23 day old rat. The patterns show continuous accumulation of lactase in proportion to aging of the differentiated enterocyte while traversing the length of the villus column. The linear patterns of lactase accumulation show a 3-fold decrease in slope from the pre- to postweaned state. It is significant that the magnitude of this decrease approximates the accompanying 3 to 4-fold decrease in the lifespan of the enterocyte. These findings are consistent with the proposal that lactase reduction in the postweaned intestine is the result of a proportional reduction in the length of stay of the enterocyte on the villus column. In Fig. 4B are shown comparative changes in protein to DNA ratios occurring across the crypt-villus column. The cells accumulate protein at a linear rate, tripling their content while traversing the villus length. Pre- and postweaned intestinal cells accumulate total protein to similar levels, in contrast to their disproportionate accumulation of lactase. These findings

indicate that synthesis of total cell protein, in contrast to lactase, occurs at an accelerated rate in the postweaned intestine.

DISCUSSION

The decline in intestinal lactase, which occurs at the normal time of weaning of the young rat, has been shown to be accompanied by a proportional reduction in the lifespan of the enterocyte. Evidence has been presented of a proportional relationship between lactase activity and the length of stay of the enterocyte on the villus column. We have proposed on this basis that reduction of the enterocyte lifespan serves as the primary determinant of enteric lactase decline in the weanling mammal. Additional evidence consistent with this proposal includes the following: rodents raised in a germ-free environment maintain elevated enteric lactase levels through adult life (19) and exhibit a corresponding marked increase in enterocyte lifespan (20). Bypassed ileum, prepared surgically in infant rats, maintain lactase at high levels beyond the weaning age accompanied by concomitant delay in reduction of enterocyte lifespan to the adult state (13). Similarly, nutritionally deprived infant rats also maintain high enteric lactase levels (21) and continue to exhibit slower infantile cell turnover rates (unpublished findings) beyond the normal weaning age.

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