Rat Milk Maintains Intestinal Lactase Activity in Rat Pups whereas Artificial Formulas Do Not

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ABSTRACT. Intestinal lactase activity is maintained at high levels in suckling rats during the first 2 wk after birth. When 12-day-old rat pups were either mother fed (MF) or artificially reared (AR) with natural rat milk or several artificial formulas, the small intestines had gained similar weight in all animal groups by 16 days except in AR rats fed a chemically defined formula. In the ileum, villus length was similar in MF and AR rats, but crypt depth was significantly higher in all groups of AR rats. Ileal absorptive cells in both MF and AR rats showed immature characteristics, including supranuclear vacuoles, apical tubular systems, and pinocytotic vesicles. Jejunal lactase specific activity and total intestinal lactase activity were significantly higher in AR rats fed rat milk than MF rats at 16 days. Ileal lactase specific activity was similar in these two animal groups. In contrast, AR rats fed artificial formulas supplemented with either glucose or lactose as the sole carbohydrate source exhibited significantly lower ileal lactase specific activity and total intestinal lactase activity than MF rats. Intestinal sucrase activity was prematurely elevated in all AR rats, even when fed natural rat milk. Addition of prolactin (3.3 μg/ml) to an artificial formula did not prevent the premature decrease in intestinal lactase specific and total activities in AR rats. We conclude that (1) natural rat milk plays a cardinal role in maintaining lactase activity during the suckling period; (2) the lactose and prolactin content of rat milk are not essential components in maintaining lactase activity in AR rat pups; and (3) the artificial feeding procedure, rather than dietary composition, induces premature elevation of sucrase activity. (*Pediatr Res* 19: 963–967, 1985)

Abbreviations

MF, mother fed
AR, artificially reared
CMF, cow milk formula
RCF, Rose carbohydrate-free formula
CDF, chemically defined formula
G, glucose
L, lactose

Intestinal lactase, which is essential for the hydrolysis and absorption of lactose, is abundant in mammals at birth, remains at high activity before weaning and then generally falls to a low level (1). In the rat, lactase activity is high for about 2 wk after

birth and declines gradually thereafter to reach adult levels during the 4th wk (2–4). In parallel, intestinal maltase activity, which is low, and sucrase activity, which is absent during the first 2 postnatal wk, increase rapidly in the end of the 3rd wk (4). Accumulated evidence indicates that the ontogenetic increase of maltase and sucrase in the rat is modulated by hormones produced by the pituitary-adrenal and pituitary-thyroid systems (5–7), and the ontogenetic decrease of intestinal lactase activity is regulated in part by the pituitary-thyroid system (8).

Previous studies from our laboratory have shown that lactase activity at 24 days postpartum is higher in rats hypophysectomized at 6 days than at 10 days of age (8), suggesting that pituitary hormones have exerted their effect on intestinal lactase activity as early as 6 days of age. Consistent with this suggestion is a report that serum concentrations of free thyroxine and triiodothyronine rise rapidly after 5 days of age (9). Intestinal lactase activity does not decline until the end of the 2nd postpartum wk (2–4, 8), so that other factors must antagonize the effect of the pituitary-thyroid system to maintain lactase activity during this period. Since prolonged nursing delays the normal decline in intestinal lactase activity (10, 11), factors in maternal milk might be responsible for maintaining lactase activity. This hypothesis is supported indirectly by our recent findings that lactase activity is precociously decreased in the ileum of artificially reared rat pups fed formula diets (12, 13).

To establish whether maternal rat milk maintains intestinal lactase activity in rat pups, we have compared the effect of natural rat milk and isocaloric formulas on intestinal lactase activity in AR rats. Since lactose protects lactase activity *in vitro* (10), and since rat milk contains high concentrations of prolactin (14) which antagonizes the effects of thyroxine on the regulation of amphibian metabolism (15, 16), studies were performed to determine whether dietary lactose or prolactin maintains intestinal lactase activity in AR rats.

MATERIALS AND METHODS

Artificial feedings. Sprague-Dawley rats, mated and bred in our animal room, had intragastric cannulae implanted at 12 days of age (17). Rats were housed individually in plastic cups containing hardwood laboratory bedding and floated in a 35° C water bath. Diets were infused continuously at rates which were increased gradually from 4.5 ml at 12–13 days to 8.0 ml at 15–16 days of age. In the first experiment, AR rats were fed either rat milk collected from dams 12–16 days postpartum, or an isocaloric carbohydrate-free soy protein formula base (RCF, Ross Laboratories, Columbus, OH) supplemented with casein hydrolysate, corn oil and either lactose (RCFL) or glucose (RCFG) to give the same caloric composition of protein, fat, and carbohydrate found in rat milk (18); a cow milk formula (CMF) (12, 19, 20); or a 14% solution of chemically defined fat-free formula (21) supplemented with either lactose (CDFL) or glucose CDFG) at the same caloric density of carbohydrate and protein as CMF.

In rats fed the defined fat-free diets, corn oil containing 2 mg of *dl-α-* tocopherol, 4 mg *dl-α*-tocopheryl acetate, and vitamins A (0.33 mg), D (0.35 mg) and K (0.55 mg) was infused separately daily (21). Some rat pups were suckled (MF) and served as controls. In the second experiment, AR rats were divided into two groups receiving either RCFL or RCFL supplemented with sheep prolactin (3.3 μg/ml, Sigma Chemical Company, St. Louis, MO). A group of MF rats served as control.

Disaccharidase assays. Animals were sacrificed at 16 days between 0900 and 1100 h. The small intestine from the ligament of Treitz to the ileocecal junction was removed, washed, blotted dry, divided into halves of equal length and weighed. The proximal half was designed as jejunum and the distal half as ileum. The intestine was homogenized in 0.15 M NaCl with a Vir Tis homogenizer (Am Sci Product, Edison, NJ). Lactase, sucrase and maltase activities were assayed by methods described previously (6–8) and protein by the Lowry technique (22). Specific activities were expressed as μmol substrate hydrolyzed per mg protein per h and total activity was expressed as μmol substrate hydrolyzed per jejunum plus ileum per h.

Histology. Morphologic observations were made in ileum since we previously showed that the premature decrease of lactase activity occurred first in the distal intestine (12). Only animals fed the diets containing lactose were studied since intestinal weight and disaccharidase activities were similar in AR rats fed lactose or glucose. For light microscopy, a segment of ileum about 3 cm from the ileocecal junction was removed, cut open, flattened on a paraffin sheet, fixed in Carnoy's fixative, and embedded in paraffin. Longitudinal sections at 5 μm thickness were stained with hematoxylin and eosin. Villus length and crypt depth were measured from 10 well-oriented villi and crypts. For electron microscopy, small segments of ileum from MF and AR rats fed either rat milk or CMF were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, at pH 7.3, overnight, postfixed in 1% OsO_4 for 2 h, and embedded in Epon. Tissues were cut at 800 Å thickness with a Porter-Blum MT-2 ultramicrotome. Sections were stained in uranyl acetate, followed by lead citrate, and observed in a Hitachi HU-12A electron microscope.

Statistical analysis. Unless otherwise indicated the results were evaluated by analysis of variance. When the F value obtained from analysis of variance was significantly different, Duncan's multiple range test (23) was then applied to test differences among groups.

RESULTS

Body and intestinal growth. AR rats infused with rat milk weighed substantially less at 16 days than MF rats (Table 1) even though the caloric intake of AR rats was calculated to approximate that of MF rats (24). Slow body weight gain also was observed in AR rats fed RCFL, RCFG, and CMF. Reduced

Table 1. *Body and small intestinal wt in MF and in AR rats (mean ± SEM)**

Group	Age (days)	No. of Rats	Body (g)	Small intestine (mg)
MF rats	12	7	23.9 ± 1.1†	774 ± 19‡
	16	6	33.5 ± 1.8	1076 ± 114
AR rats + RM	16	5	27.4 ± 1.0‡	971 ± 42
+ RCFL	16	5	27.0 ± 0.6‡	1057 ± 38
+ RCFG	16	5	28.4 ± 0.9‡	1042 ± 49
+ CMF	16	6	27.0 ± 0.5‡	1049 ± 32
+ CDFL	16	5	24.8 ± 1.2†	837 ± 26‡
+ CDFG	16	5	24.6 ± 0.7†	817 ± 49‡

* Rat pups at 12 days of age were MF or AR with either rat milk (RM), RCFL, or RCFG, a CMF, or a chemically defined formula supplemented with lactose (CDFL) or glucose (CDFG).

† $p < 0.01$ and ‡ $p < 0.05$ compared with MF rats at 16 days.

Table 2. *Villus length and crypt depth in the ileum of MF and AR rats on differing diets (mean ± SE)*

Groups	No. of rats	Villus length (μm)	Crypt depth (μm)
MF rats	5	282 ± 7	42 ± 1.9†
AR rats + RM	3	283 ± 9	65 ± 7.6
+ RCFL	5	269 ± 12	70 ± 3.7
+ CML	4	310 ± 23	72 ± 4.3
+ CDFL	4	262 ± 9	63 ± 4.2

* Rat pups were MF or AR with rat milk (RM) or artificial formulas from 12 days of age and were sacrificed at 16 days.

† $p < 0.01$ *versus* other values in the same column.

weight gain was not due to changes in the gastric emptying rate since the weight of gastric contents were similar in all AR groups (114–210 mg). However, at the same time the small intestine weight gain in AR rats fed either RM, RCFL, or CMF was comparable to that of MF rats (Table 1). The perfusion rate in AR rats was chosen to maintain similar intestinal weights since the objective of the present studies was to compare intestinal enzyme contents. If sufficient calories are provided to AR rats to maintain normal body weight gain, intestinal hyperplasia occurs (12, 13). Intestinal weight did not increase only in AR rats fed CDFL or CDFG. Addition of prolactin into RCFL had no promoting effect on small intestinal or body weight gain (data not shown).

Ileal morphology. At 16 days villus length in MF and AR rats fed either rat milk or artificial formulas containing lactose was similar, but crypt depth in MF rats was significantly lower than that in AR rats (Table 2). No significant difference in crypt depth was found among groups of AR rats.

Ileal morphology was similar in MF and AR rats. Figures 1 to 3 show that epithelial cells covering the upper three-quarters of villi were highly vacuolated in MF rats, in milk fed AR and in CMF fed AR rats. Similar morphology was also found in other AR rats fed RCFL and CDFL diets (data not shown). Ultrastructural studies indicated that the absorptive cell at the middle villus level exhibited one large supranuclear vacuole and associated small vacuoles and apical tubular systems in MF rats (not shown) and AR rats fed rat milk or CMFL (Figs. 4 and 5). The presence of pinocytotic vesicles in absorptive cells implies that these cells are actively taking up luminal nutrients.

Intestinal disaccharidase activities. Lactase specific activity in MF decreased by 28% in the jejunum but did not change in the ileum (Fig. 6 *a* and *b*, *open columns A* and *B*). Jejunal lactase activity at 16 days of age in AR rats fed rat milk remained at the 12-day-old level (*columns A* and *C*), and was higher than that of 16-day-old MF rats. In AR rats fed diets other than rat milk, jejunal lactase specific activity decreased to the levels found in MF at 16 days. In contrast to the findings in the jejunum, ileal lactase activity dropped prematurely in rat pups given diets other than rat milk (Fig. 6*b*, *columns D, E, F, G,* and *H*).

Although lactase specific activity of jejunum fell, total lactase activity in MF (Fig. 6*C*, *open columns A* and *B*) increased significantly between 12 and 16 days because intestinal weight rose by 39%. Total lactase activity in AR rats fed rat milk at 16 days was also higher than in MF rats of the same age (*columns B* and *C*). However, increased lactase activity occured only in rats provided with rat milk and enzyme activity actually decreased in AR rats fed other diets ($p < 0.05$).

In studies on sucrase and maltase activity of AR rats performed in parallel, both specific and total activities of these enzymes were prematurely elevated in the jejunum and ileum (Fig. 6, *cross-hatched columns* show sucrase activity, maltase activity was similar and is not shown). In MF rats no sucrase activity could be detected at 12 days, and only two of six rats showed low levels of activity at 16 days (Fig. 7, *column B*).

When AR rats were fed RCFL plus prolactin, both specific

and total intestinal lactase activities declined to levels significantly lower than those of MF rats (Table 3, $p < 0.01$). Specific lactase activity in both jejunum and ileum and total lactase activity in AR rats fed RCFL plus prolactin were not significantly higher than those in AR rats fed RCFL only ($p > 0.05$).

DISCUSSION

Since body weights of AR rats did not increase as much as MF rats, it was conceivable that malnutrition, possibly resulting from inadequate absorption of nutrients, was responsible for our ob-
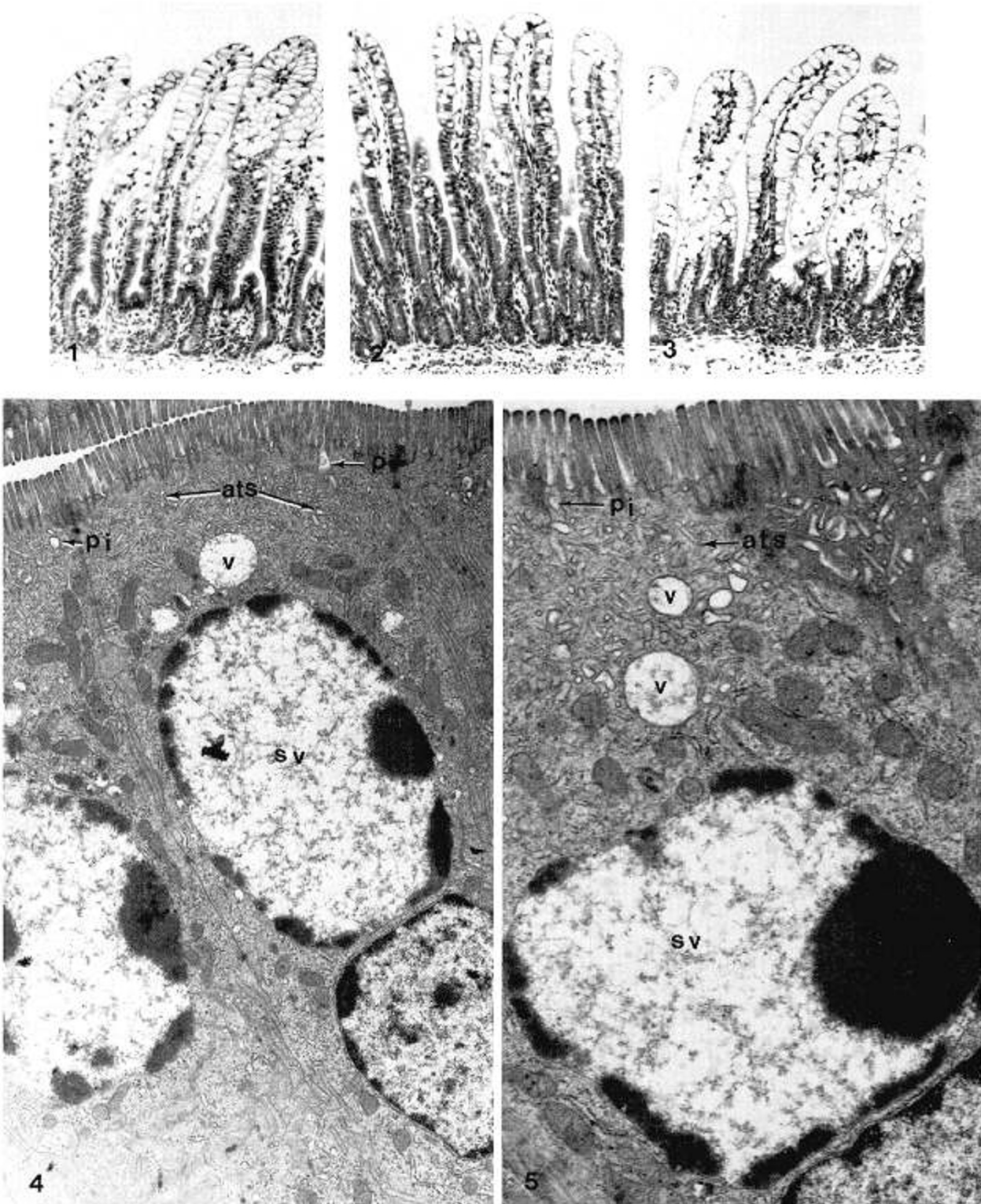
Figs. 1–3. Light microscopy of ileal villi in MF rats (*1*) and in AR rats fed either rat milk (*2*) or CMFL (*3*). Vacuolated cells cover the upper three quarters of villi in all animal groups. ×110.

Figs. 4 and 5. Electron micrographs of ileal absorptive cells in AR rats fed rat milk (4,000 × 5,000) or CMFL (5,000 × 10,000). Absorptive cells show immature characteristics including pinocytotic invaginations and vesicles (*p*), apical tubular systems (*ats*), numerous small vacuoles (*v*), and a large supranuclear vacuole (*sv*).
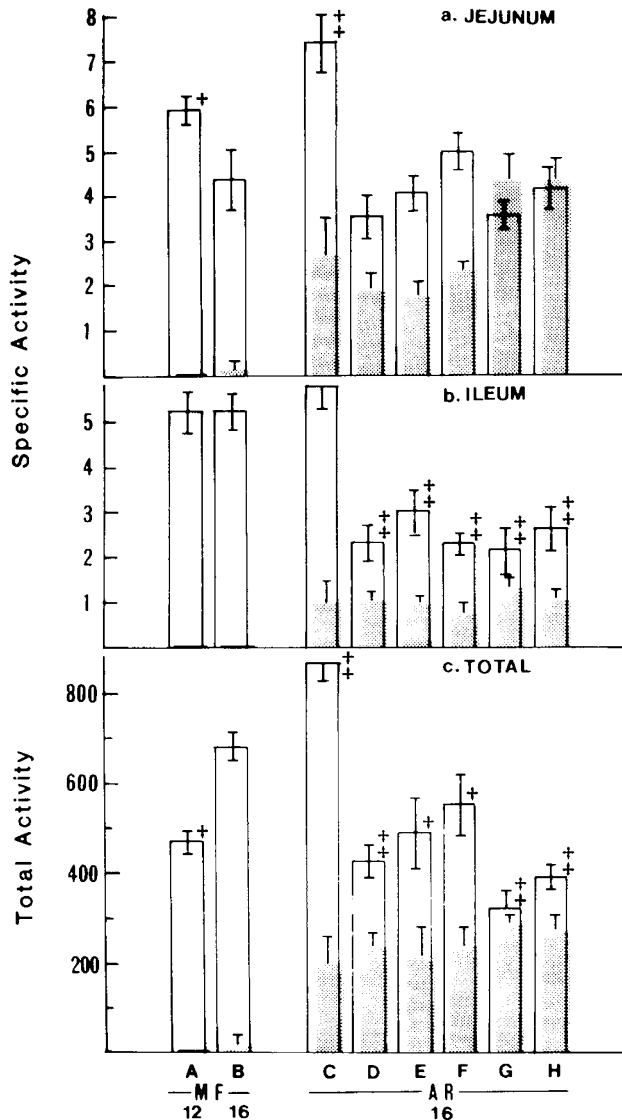
Fig. 6. Specific activities of lactase (*open columns*) and sucrase (*cross-hatched columns*) in the jejunum (*a*) and ileum (*b*) and total intestinal lactase and sucrase activities (*c*). Rat pups at 12 days of age were MF or AR with either rat milk (*column C*), RCFL (*column D*), RCFG (*column E*), CMF (*column F*), CDFL (*column G*), or CDFG (*column H*) from 12 to 16 days. Each *column* represents the mean of five to seven animals. *Vertical lines* indicate SEM. For the probability of lactase activity. †*p* < 0.05 and ‡*p* < 0.01 compare with MF rats at 16 days (*column B*).

servation. However, our present studies demonstrated that epithelial cells in AR rats retained high pinocytotic activity. Furthermore, ileal villi of AR rats were not shorter than those of MF rats. These morphologic characteristics suggest normal intestinal digestive functions in AR rat pups. It is likely that undernutrition of AR rats is the result of a low caloric intake since higher caloric intake is able to support AR rats to gain weight comparable to MF rats (12, 13).

Malnutrition and starvation have been shown to elevate intestinal lactase specific activity, but have no effect on total activity (25, 26). In the present studies both lactase specific and total activities were increased in AR rats fed rat milk implying that the changes observed cannot be attributed to malnutrition only. Similarly, caloric undernutrition could not have caused the precocious decline in ileal lactase specific activity, since this occurs in AR rats provided with an even higher caloric intake of CMF and RCFL (13).

Lower rates of body weight gain may reflect the effects of stress. Our previous results suggested that changes in intestinal sucrase activity were not induced by the diet (12, 13), but appeared to be an effect of stress (25, 27, 28). This interpretation is supported by recent data in our laboratory showing that serum corticosterone concentrations 24 h after cannula implantation were 5.30 ±0.67 μg/dl in AR rats fed RCFL diet and 0.47 ± 0.21 μg/dl in MF rats. In contrast to suckling rats, dietary sucrose enhances intestinal sucrase synthesis in adult rats (29). All AR rats in the present studies showed a precocious increase in sucrase (and maltase) independent of the substrate fed. However, stress was not responsible for the decreases in ileal lactase activity since this was maintained by rat milk in AR rats.

The maintenance of high lactase activity in AR rats fed rat milk is consistent with observations that prolonged nursing delays the usual decrease of lactase activity (10). As a result of higher jejunal lactase activity, total lactase activity was significantly higher in AR rats fed rat milk than MF rats. Since a small amount of Purina Chow was present in the stomach of MF rats at 16 days, this partial weaning might be responsible for decreased lactase activity. A precocious decrease of ileal and total intestinal lactase activity occured in AR rats fed RCFL, a formula in which the lactose content closely simulates that of rat milk. Ileal lactase activity also decreased in AR rats fed CMF indicating that evaporated cow milk, which contains 50% higher lactose concentration than rat milk (12), is unable to prevent the fall in lactase activity. Furthermore, ileal and total intestinal lactase activity fell to the same extent if AR rats were fed artificial formulas containing isocaloric amounts of either lactose or glucose. All of these studies demonstrate unequivocally that the presence of lactose in the milk formula does not maintain intestinal lactase activity.

The precise mechanism for the premature decrease in ileal lactase activity in AR rats fed artificial formulas when compared to rats fed rat milk is unknown. Changes in lactase activity have been shown to be closely related to the turnover rate of intestinal epithelial cells (30). If AR rats fed formulas were to have greater epithelial cell turnover rates than rats fed rat milk, cells with high sucrase and low lactase activity, usually found near the intestinal crypt, conceivably might replace those cells with high lactase activity near the villus tip. Studies of cell turnover rates in response to different diets in AR rats are in progress.

Intestinal lactase activity is more susceptible to protease degradation than sucrase activity (31), so that low ileal lactase activity might have resulted from increased luminal concentrations of proteases. Increased bacterial protease concentrations might possibly be present in AR rats fed artificial formulas since maternal milk contains antibacterial factors that are not present in artificial formulas (32, 33).

In addition to antimicrobial factors and nutrients, rat milk contains several hormones, growth factors, and hormone-binding proteins (33–36). It is likely that one or more of these biologically active components in maternal milk has a role in sustaining intestinal lactase activity during the suckling period. Since rat milk contains high titers of prolactin (14) and since prolactin

Table 3. *Effect of prolactin on intestinal lactase activity in AR rats (mean ± SE)**

Group	No. of rats	Specific activity (μmol/ mg protein/h) Jejunum	Ileum	Total activity (μmol/ intestine/h)
MF rats	5	4.15 ± 0.44	5.16 ± 0.19	587 ± 64
AR rats + RCFL	6	2.25 ± 0.41†	1.89 ± 0.41†	254 ± 44†
+ RCFL + PRL	7	2.87 ± 0.23†	2.65 ± 0.19†	381 ± 53†

* Rats at 12 days of age were either MF or AR with RCFL or RCFL plus prolactin (PRL) and were sacrificed at 16 days.

† *p* < 0.01 *versus* MF rats.

antagonizes the effect of thyroxine on amphibian metamorphosis (15, 16), it might similarly antagonize the suppresive effect of thyroxine on intestinal lactase activity in suckling rats. The present results however, show that sheep prolactin at a concentration 10 times higher than in rat milk (14), has no effect on sustaining intestinal lactase activity. Further studies of feeding AR rats with a formula supplemented with other components in milk will be needed to define the specific components responsible for maintaining intestinal lactase activity during this important postnatal period.

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