Pediat. Res. 3: 27–33 (1969)

Developmental biochemistry
hydrocortisone
intestine
invertase
nutrition
weaning

Postnatal Development of the Small Intestine of the Rat

Changes in Mucosal Morphology at Weaning

JOHN J. HERBST[26] and PHILIP SUNSHINE

Department of Pediatrics and Human Development and the Lt. Joseph P. Kennedy, Jr., Laboratories for Molecular Medicine, Stanford University School of Medicine, Palo Alto, California, USA

Extract

At 15 days after birth, the small intestine of the rat showed a striking increase in relative weight, an increased depth of intestinal crypts, an elevation of the mitotic and the labeling index of crypt cells, and a decrease in the ratio of height of the villi to depth of the crypts.

Metabolically, the biological half-life of ^{14}C phenylalanine incorporated into protein was decreased in the intestine of 21-day-old rats compared with that of the 32-day-old rat. With autoradiography, a decreased half-life of ^{3}H-leucine in crypt cells was noted in rats 5 and 21 days of age, compared with fully weaned rats 32 days of age. These observations indicated that protein turnover is not completely dependent on cellular turnover in suckling rats.

Artificial feeding of suckling rats from 9 to 15 days of age produced an increase in the relative weight, mitotic index, depth of crypts, and activity of invertase in the jejunum. Prolonged administration of hydrocortisone to newborn rats resulted in similar changes in the duodenum and in the jejunum, but not in the ileum. These changes could be related to an increased rate of cellular migration along the villi of the duodenum and the jejunum. It was concluded that both diet and hormones were important factors in the marked changes in intestinal cell proliferation and differentiation observed at weaning.

Speculation

The usual changes in cellular proliferation and differentiation of the intestinal mucosa of the weanling rat and the observation that these changes can be stimulated precociously suggest that the intestinal tract of suckling rats could be used to investigate further those factors important to the control of cell differentiation. Since cell turnover increases to adult levels after 15 days of age, the observed increase in protein turnover in suckling and in weanling rats, compared with that of adult rats, suggests that at these ages protein turnover is not coordinated with cell turnover.

Introduction

Cellular proliferation has been studied extensively in animals and in man, usually in the adult [4, 14, 16]. When the rat is weaned, the intestine undergoes remarkable morphological and chemical changes. The rate of migration of epithelial cells along the villus in the sucking rat is only one-fourth to one-fifth that of the adult animal, but increases during weaning to rates observed in the adult intestine [12]. Concomitantly,

there is a considerable increase in activities of alkaline phosphatase [17], maltase [20], and invertase [6] in the intestinal mucosa and a cessation of pinocytotic activity of mucosal cells [5].

Intestinal crypt cells are constantly proliferating and differentiating. The dramatic cellular changes that occur during the process of weaning prompted this investigation of cellular growth and kinetics in the intestine of developing rats, and an attempt was made to assess those regulatory factors that might participate in these changes. Since several of the changes in the changes in the intestine that occur at weaning can be precociously induced by steroids [5, 6, 17], administration of hydrocortisone and premature weaning were factors used to regulate the onset of maturation of the intestine.

Methods

Intestinal Growth

Wister rats were used in this study. To reduce variability, the size of the litter was reduced to eight animals on the second day of life [11]. Following decapitation, the intestine was washed with ice cold 0.15 M potassium chloride. The small intestine from the ligament of Treitz to the caecum was weighed and correlated with the weight of the animal. The weight of jejunum and ileum was determined in 141 rats using a minimum of four animals at each age studied.

For histological examination and preparation of histoautoradiographs, samples of duodenum, jejunum, and ileum were fixed in formalin prior to processing. Multiple histological sections were scanned for areas in which the section passed through the entire vertical length of a villus and crypt. In these areas, the height of the villus and the depth of the crypt column were determined in 10 villi and crypts per organ. To minimize errors caused by shrinkage of tissues during preparation of the sections, distances were measured by counting the number of cells lining one side of a villus or one side of a crypt column under 640 × magnification.

The rates of cellular proliferation of the intestine at different ages were compared by determining the percentage of cells undergoing mitosis at any one time (mitotic index), and by comparing the percentage of cells undergoing DNA synthesis demonstrated by incorporation of tritiated thymidine into nuclear material (labeling index). In each organ, one thousand crypt cells were counted under oil immersion to determine the mitotic index; similarly, the labeling index was determined four hours after intraperitoneal administration of tritiated thymidine by means of autoradiography [13]. The mitotic index and the labeling index were determined in at least 10 and 7 animals, respectively, in each age group.

Protein turnover was studied in whole mucosa by intraperitoneal injection of uniformly labeled ^{14}C phenylalanine (>300 μc/mM) into litters of rats 21 days of age (0.02 μc/g) and 32 days of age (0.04 μc/g). The animals were killed at intervals up to 96 hours after injection; mucosa was removed by gently scraping with a stainless steel spatula, weighed, and homogenized in 0.15 M KCl in a Potter-Elvehjem homogenizer with a Teflon pestle. Aliquots of this homogenate were precipitated in 10 % trichloroacetic acid; the precipitate was collected on glass fiber filter discs according to the method of NIRENBERG [18]. The discs were counted in a Packard Tri-Carb liquid scintillation counter using 0.4 %, 2,5 diphenyloxazole (PPO) and 0.01 %, 1,4-bis-(2-[4 methyl-5-phenyloxazolyl])-benzene (POPOP) in toluene as the scintillation fluid. All counts were at least ten times background; quenching was determined by the channels ratio method. Results were expressed as counts per minute per gram wet weight of mucosa.

Protein turnover in crypts was determined by injecting 2 μc/g leucine 4,5 ^{3}H (5 c/mM) intraperitoneally into litters of rats of 5, 21, and 32 days of age. Animals were killed at intervals, and autoradiographs of the jejunum and the ileum were prepared by the method of KOPRIWA and LEBLOND [13]. The grain density in the emulsion overlying the crypts was determined by counting the average number of silver grains in ten areas of crypt cells and subtracting background fog. Oil emersion magnification and an eyepiece grid that outlined an area of 500 μ^2 were used.

Precocious Maturation of Intestine

A functional maturation of the intestine occurs at weaning. Several changes can be precociously induced by administration of hydrocortisone [5, 6, 17]. The effects of early weaning and hydrocortisone on several parameters of cellular proliferation were investigated.

Early Weaning. Nine-day-old rats were prematurely weaned from mothers milk and fed by gavage an artificial formula composed of homogenized cows milk supplemented to 8 % protein and fat with lactalbumin and corn oil. Part of the litter remained with the mother to serve as a control. The rats were fed every three hours the first 24 hours, then every four hours; the volume of the feeding was empirically adjusted so that some milk remained in the stomach at the next feeding. Between feedings, the rats were kept at an ambient temperature of 32 ± 1° with the aid of an electric lamp. Since young rats separated from the mother may fail to defecate or urinate, these functions were readily stimulated by gently stroking the abdomen. The above study was also repeated using nonlactating female rats as mother substitutes, and identical results were obtained.

Hydrocortisone Administration. Three-day-old rats were injected subcutaneously with hydrocortisone emulsion [23], 25 mg/kg each day and compared with saline-injected control littermates at 11 days of age. The rate of cellular migration along the villus was determined by a method reported previously [12], and the activity of invertase, using glucose oxidase, was determined by the method of DOELL and KRETCHMER [6].

Results

Normal Development

After 15 days of age, there was a 60 % increase in the relative weight (weight as a percent of body weight) of the jejunum and the ileum (fig. 1), in comparison with the value during the first 10 days of life. The increased relative weight of these organs in rats 21–25 or 26–30 days of age was significantly greater than that in rats 6–9 or 11–15 days of age, $p = 0.001$.

This growth spurt was reflected in the cellular components. The mitotic index in animals over 21 days of age was significantly greater than that in animals 6–10 or 11–15 days of age, $p = 0.001$ (fig. 1). The labeling index increased from 27.3 ± 1.7 % in suckling rats less than 15 days of age to 39.5 ± 3.1 % in rats more than 21 days of age. The observed increase in both the mitotic index and in the labeling index strongly suggests an increase in the rate of cellular proliferation.

The relative proportion of the differentiated nonproliferating villus cells, compared with the actively dividing crypt cells, is indicated by a ratio of the height of the villus to the depth of the crypt column. Although the height of the villus increased during the first month of life, the most marked change was a 2 ½-fold increase in the depth of the crypt columns (fig. 2).

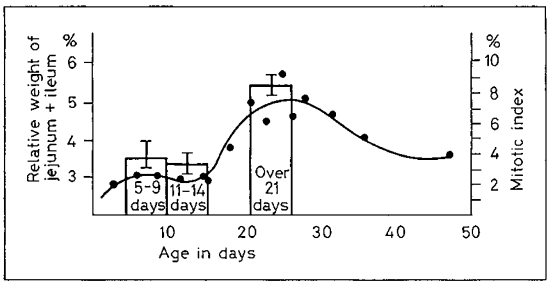


Fig. 1. The relative weights of the jejunum+ileum (weight of jejunum+ileum/body weight × 100) are plotted as individual points. Each point represents at least 4 animals. The mitotic index at different ages is represented by a bar graph. Vertical lines represent one standard deviation.

Protein Turnover

The biological half-life (t ½) of ^{14}C phenylalanine in whole intestinal mucosal homogenate of the rat at day 32 was 60 hours and at day 21 was 36 hours (fig. 3). The t ½ of ^{3}H leucine in crypt cells in the rat at day 32 was 22 hours; at day 21, 11 hours; and at day 6, 15 hours (fig. 4).

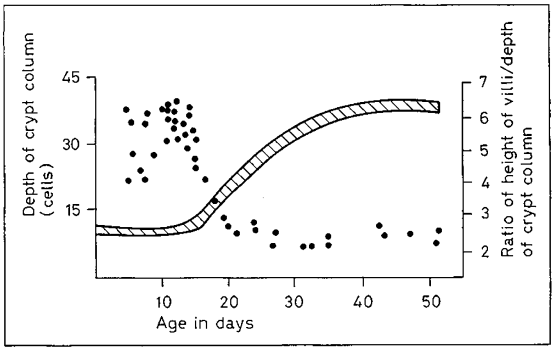


Fig. 2. The depth of the jejunal crypt column at different ages is plotted as the shaded area, which represents the range encountered. The ratio of the height of the villus/depth of crypts in the jejunum is plotted as individual points.

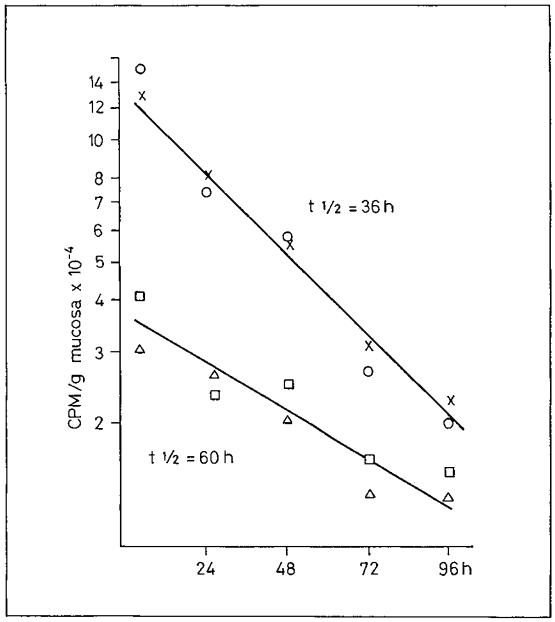
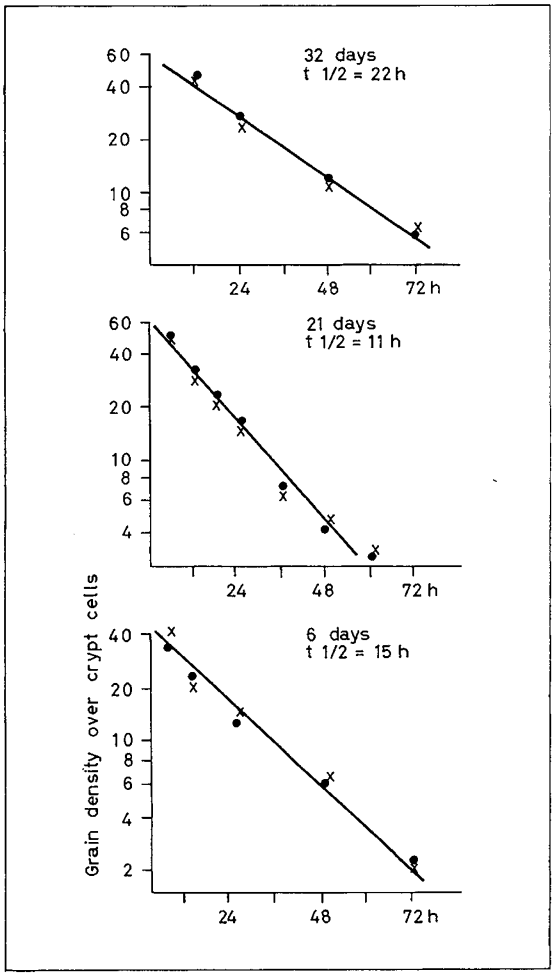


Fig. 3. Activity of ^{14}C phenylalanine incorporated into protein derived from jejunum and ileum of 21- and 32-day-old rats. t ½ is the biological half-life in 21-day jejunum (○); 21-day ileum (×); 32-day jejunum (△); 32-day ileum (□).



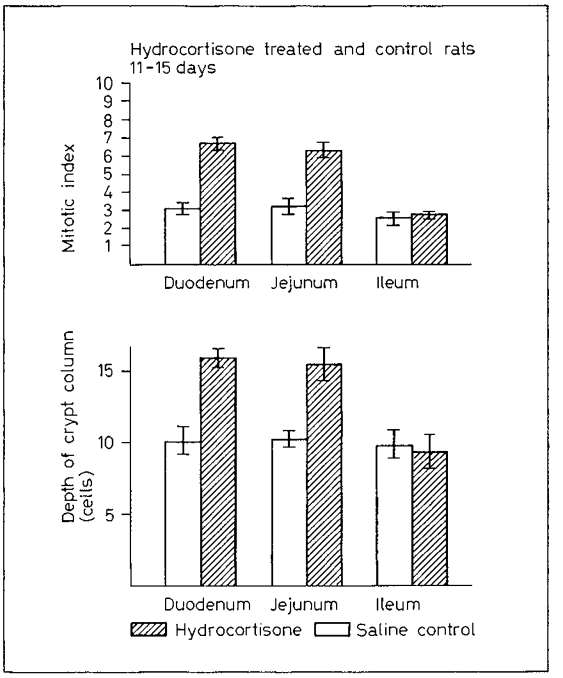


Fig. 5. Mitotic index and depth of crypt column in different areas of the small intestine in hydrocortisone-treated (cross-hatched) and saline-injected control rats at 11 days of age. The changes in duodenum and jejunum are significant ($p < 0.001$). Vertical lines indicate standard deviation.

◀ *Fig. 4.* Grain densities (grains per 500 μ^2 area) over crypt cells of 6-, 21-, and 32-day-old rats. t ½ is the biological half-life.

Table I. Effect of diet on intestinal maturation of rats fed day 9 to day 15

Animal	Body weight g	% body weight jejunum+ileum	Depth of jejunal crypt column in cell number	Mitotic index	Invertase activity[1]
Control	25.5	3.08	12.3	3.4	0.00
	25.7	3.09	12.6	3.4	0.00
	25.5	2.93	12.3	3.5	0.00
	26.2	2.90	12.1	2.7	0.00
Sham fed	25.3	3.09	12.1	3.2	0.00
	26.7	2.74	12.0	3.0	0.00
	27.0	3.08	12.2	3.0	0.00
	26.0	3.18	12.3	3.0	0.00
Fed	24.3	4.90	17.0	6.2	8.33
	24.5	4.42	16.1	6.7	6.06
	24.0	4.42	16.0	6.4	6.25

[1] μmol substrate consumed/min/g wet weight of tissue.

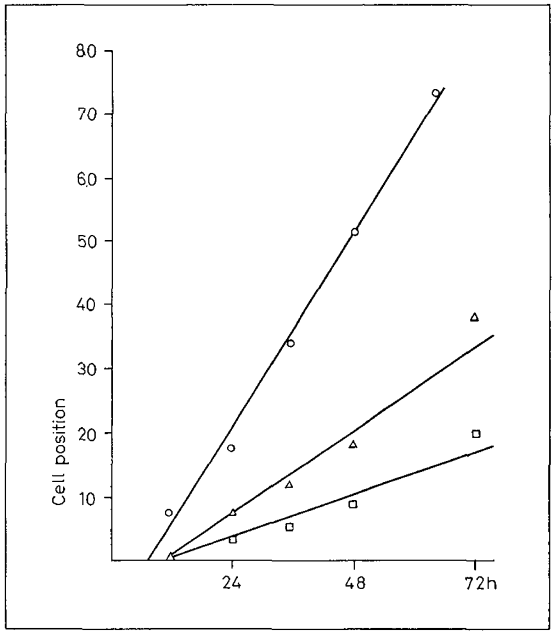


Fig. 6. Rate of migration of leading edge of thymidine-labeled cells along the villus of duodenum and jejunum (○) and ileum (△) of hydrocortisone-treated rats and in all areas of control rats (□). Each figure represents at least 2 animals.

Induction of Precocious Maturation of Intestine

Rats from 9 to 15 days of age, fed artifically, had a weight gain similar to that of sham-fed controls, but showed an increased relative intestinal weight, mitotic index, and depth of jejunal crypt columns (table I). Invertase activity was precociously induced in the artificially fed rats 15 days of age. Identical results were obtained in those artificially fed rats that were mothered by a nonlactating female.

Three-day-old rats received hydrocortisone [23] by subcutaneous injection, 25 mg/kg each day, and were studied at 11 days of age. Although the rats that received hydrocortisone were stunted when compared with their littermates that were given saline injections, the intestines of the rats treated with hydrocortisone had an increased depth of crypt column and an increased mitotic index in the duodenum and in the jejunum (fig. 5). This increase correlated with a four-fold increase in the rate of cellular migration along the villus (fig. 6). Minimal changes were noted in the ileum. Administration of hydrocortisone to newborn rats for three to six days or to 12-day-old rats for three days did not produce the changes mentioned above, although invertase activity was induced [6]. Administration of hydrocortisone to suckling rats for short periods induced invertase activity, but injection of hydrocortisone into newborn rats for three or six days, or into 12-day-old rats for three days, did not cause mucosal hypertrophy associated with continuous administration of hydrocortisone from the third to the eleventh day.

Discussion

Usually, infant rats subsist entirely on maternal milk until 12 to 13 days of age and then start to nibble other foods. The infant animals may be removed safely from the mother at 21 days of age and will have ceased to suckle by about 28 days of age. The weaning period is considered to extend from the twelfth to the twenty-eighth day of life.

After 16 to 19 days of age, there is a marked increase in the activity of several enzymes in the intestinal mucosa [6, 17, 20]. This study demonstrated that at weaning there is an increase in the relative weight of the intestine, a relative decrease in the population of villus cells when compared with the dividing crypt cells, an increase in the depth of the crypt column, and an increase in the mitotic index and in the labeling index of crypt cells. These changes occurred after the fifteenth day of life. The data indicate that at the time of weaning there is an increase in the absolute and relative population of proliferating cells and in the rate of proliferation of crypt cells. This proliferation correlates with a four-fold increase in rate of cellular migration along the villus in weaned animals as previously reported [12]. ALTMAN and ENESCO [2] have shown that at an age when the transition from the suckling to the weaned appearance of the intestine is being initiated (18 days), the rate of cellular proliferation in the intestinal crypts is less than that in fully weaned or adult rats.

The t ½ of radioactive amino acids incorporated into protein of the whole mucosa or into crypt cells of 32-day-old rats is similar to published values for the adult mouse in which the disappearance of label from the crypts is caused by emigration of cells [15].

The turnover rate of mucosal cells is four times greater in 32-day-old rats than it is in five-day-old rats, but the turnover of tritiated leucine in crypt cell proteins is more rapid in the five-day-old rat. The rate of cell turnover is similar in the intestines of 21- and 32-day-old rats, but the rate of protein turnover in either crpyt cells or in whole mucosa is more rapid in the 21-day-old rat (two times as rapid in crypt cells and one and one-half times as rapid in whole mucosa, as indicated by the half-lives of amino acids in intestinal proteins). Thus, in the young rat, the rate of protein turnover in intestinal mucosa cannot be correlated with cell turnover as it can be in the adult [15].

The mechanism that causes the increase in cellular proliferation at weaning is unknown. Intestinal hypertrophy has been induced in hyperphagic rats [22] and in rats following intestinal resection [7]. Also, intestinal weight and cellular proliferation increase in the lactating rat [3], reaching a peak two weeks after parturition; these then subside at a time when cellular proliferation increases in the suckling offspring. These findings suggest that dietary or hormonal changes may be important factors in the control of intestinal cell proliferation and differentiation at weaning.

In order to investigate the effect of diet, rats were fed by gavage a cows milk formula, supplemented to resemble rat milk [8]. The amount of the gavage was adjusted so that the stomach was never empty, since frequency of feeding has been shown to affect intestinal weight in adult rats [9]. If changes toward adult values in the relative weight of the intestine, mitotic index, depth of crypt column, and the appearance of sucrase activity may be considered maturation, artificial feeding produced a precocious maturation in the jejunum in all parameters measured.

The similarity of the crypts in suckling and in germfree rodents is striking [1], but the intestine of the suckling rat is not sterile [21]. Daily gavage of a fecal suspension to suckling rats failed to induce precocious development of the intestine [10], suggesting that the mechanism by which the artificial weaning from maternal rat milk caused maturation of the intestine was not merely the introduction of bacteria into the intestine.

The finding that a single dose of hydrocortisone induces activity of several enzymes in the intestinal mucosa of suckling rats suggested that hydrocortisone might be effective in inducing an increase in cellular proliferation. In preliminary experiments, injection of hydrocortisone into newborn rats for three to six days, or into 12-day-old rats for three days, produced no effect upon crypt size or mitotic index. When injected into suckling rats for nine days, hydrocortisone caused a maturation of the size of the crypts and mitotic index in the duodenum and in the jejunum, but not in the ileum.

Prolonged administration of steroids to adult mice, a species that also has an intestinal growth spurt at weaning [19], does not significantly alter cell kinetics in the jejunum [15]. These data suggest that the effects of steroids on the adult intestine may be maximal, or that steroids exert action during a critical period in development. The data also indicate that control of cellular proliferation may be different in the ileum than in the duodenum and jejunum of suckling rats, even though the mitotic rates are similar in the adult in all regions of the small intestine [14].

Summary

In rats, the intestines undergo a growth spurt at weaning. The actual and relative size of the crypts, as well as the mitotic and the labeling index, increases to adult levels after 15 days. There is an increased rate of protein turnover in the intestine of the suckling rats, demonstrated by radioautography of crypt cells or measured in trichloroacetic acid precipitable protein from whole mucosal homogenates.

Precocious maturation of the jejunum can be induced by the feeding of an artificial formula. Prolonged administration of hydrocortisone to suckling rats will also cause precocious maturation in the duodenum and in the jejunum, but not in the ileum. These changes were correlated with an increased rate of cellular migration along the villus.

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24. We should like to thank Miss Rosa Medina for her expert technical assistance.
25. This work was supported in part by Research Grants HD 02147, HD 00391, and HD 0049 from The National Institutes of Health, USPHS, and by a grant from the National Foundation.
26. Requests for reprints should be addressed to: JOHN J. HERBST, M.D., Research Fellow in Pediatrics, Department of Pediatrics and Human Development, Stanford University School of Medicine, Palo Alto, Cal. 94304 (USA).