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Changes in Upper Intestinal Epithelial Morphology and Kinetics in the Growing Guinea Pig

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ABSTRACT. Weaning, the process of intestinal adaptation from milk to solid diet, demands changes in gastrointestinal function. We aimed to measure upper intestinal mucosal morphology and cytokinetics during early life, to determine whether the marked changes seen at weaning in altricial species, such as rat and mouse also occur in the precocial guinea pig. A total of 79 animals was studied. Jejunal morphology was measured by microdissection and crypt cell production rate by a metaphase arrest technique in animals at 1, 7, 14, 21, and 28 days after birth. There was a 40% decline in villus height from 986 to 576 μm during the first 2 wk ($p < 0.001$). Crypt depth increased by 25% from 148 to 199 μm ($p < 0.01$). After an initial decline there was a significant increase in crypt:villus ratio from 6.7 to 8.2 ($p < 0.001$), in crypt cell production rate from 3.9 to 5.6 cells/crypt/h ($p < 0.001$), and net villus influx from 26.1 to 45.9 cells/villus/h ($p < 0.001$) from 14 days onward. These proliferative changes were accounted for by an increase in the depth and number of crypts, and in crypt cell production rate, leading to an increase in net villus influx. In contrast with the rat and mouse they were gradual, occurred largely during the 3rd wk, and appeared to follow the cessation of breast feeding and commencement of solid food. It is suggested that the functional changes in the small intestine that occur during weaning in the guinea pig are neither due to rapid proliferation of a new epithelial cell population, nor precipitated by change in diet. (*Pediatr Res* 26: 31–33, 1989)

Abbreviations

C:V, crypt:villus ratio
CCPR, crypt cell production rate

There are two important periods in the gastrointestinal adaptation of the newborn mammal to enteral nutrition: birth and weaning. Those changes which occur perinatally have been well studied, particularly in rodents (1, 2). They are largely concerned with the transition from intrauterine parenteral nutrition to extrauterine enteral nutrition. Weaning, the period of transition from milk to solid feeding, has also been the subject of studies in the rat and mouse (2), but little is known of the process in other rodent species, including the guinea pig.

There is a marked difference in the relative maturation of these species at birth. The newborn rat and mouse are born after a short gestation, closely dependent on their dams for nutrition, immunoprotection, thermoregulation, locomotion, and help in evacuation of bowels and bladder. Not until after weaning do they achieve independence. During the suckling period they require milk high in protein, rich in immunoglobulin and containing trophic factors which may contribute to perinatal gastrointestinal adaptation (1). Postnatal growth is rapid. Major changes in gastrointestinal mucosal morphology and function (2, 3) occur during weaning. These are accompanied by a sharp increase in small intestinal epithelial cell production rate (4–6).

The guinea pig, born after a long gestation, is relatively mature at birth, with fur, teeth, autonomous thermoregulation, and locomotion. It is less dependent on maternal milk for either nutritional or non-nutritional purposes, and undergoes changes in mucosal architecture (7), enterocyte morphology (8), and function (7, 9) in the immediate neonatal period. It may be reared on an artificial milk formula, and can consume solids soon after birth (7, 10). Intestinal closure to macromolecular uptake occurs within a few days of delivery (11). Postnatal growth is slow (7, 10).

The purpose of our study was to measure the changes in upper intestinal epithelial architecture and cytokinetics during early postnatal life in a species which is precocial at birth to test the hypothesis that, in contrast with altricial species, abrupt changes in gastrointestinal structure and function do not occur at weaning. In the precocial guinea pig the perinatal period is the major time of postnatal gastrointestinal adaptation, and is followed by a gradual change in epithelial morphology and cytokinetics during and after transition from milk to solid feeding.

MATERIALS AND METHODS

A total of 79 outbred Dunkin Hartley guinea pigs aged 1 to 28 d was studied (Table 1). Animals remained with their dams until weaned, after which they were caged in pairs. Animals of all ages were allowed access to solid diet.

A stathmokinetic method was used to measure intestinal epithelial turnover (12). Experiments were performed in the morning. Each guinea pig was given colchicine 7 mg/kg body wt by intraperitoneal injection. Animals were killed at 20 or 30 min intervals thereafter by cervical dislocation. The abdomen was opened by longitudinal midline incision and segments of small intestine of one cm length were excised 5 cm distal to the pylorus, opened longitudinally and laid, mucosal side up, on card before fixation in Clarke's solution (75% ethanol, 25% acetic acid).

Microdissection. After fixation for 24-h specimens were stored in 75% ethanol until staining with Schiff reagent by the Feulgen reaction. This was preceded by rehydration through descending concentrations of ethanol and hydrolysis with 1 M HCl at 60°C. The serosal and muscle layers were removed and individual crypt-villus units were dissected under a stereomicroscope at ×20

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magnification. These were laid on a microscope slide under a cover slip in 2 to 3 drops of 45% acetic acid and the following measurements were made.

Villus height and crypt depth. The heights of 10 well-orientated villi, and the depths of 10 well-orientated mature crypts per animal were measured using an eye-piece micrometer.

C:V ratio. The ratio of the number of crypts to each villus was measured by examining pieces of undissected mucosa, stripped of their serosal and muscle layers, mounted between two microscope slides in 45% acetic acid. Two to six specimens per animal were analyzed. The C:V ratio was calculated by dividing the number of crypts by the number of villi per unit area using an eye-piece grid.

CCPR. Gentle pressure was applied to the cover slip to produce squash separation of individual crypts. The number of well stained metaphase arrest figures per 10 complete undamaged crypts per animal was measured. CCPR was calculated from the slope of the plot of the mean number of metaphase arrest figures per crypt per animal against time after administration of colchicine (4, 12).

Statistics. The results were expressed as means and SEM. Litters of at least three animals were used for the calculation of CCPR. The significance of the changes in results with age was analyzed using an unpaired *t*-test for the differences between individual days, and one-way ANOVA for changes over time.

RESULTS

Growth. Steady postnatal growth of animals was observed at approximately 25 g/day, with a doubling of birth wt within the first 2 wk (Table 1).

Villus height. There was a 40% decline in villus height during the first 2 wk from 986 to 576 μm ($p < 0.001$), but no significant change thereafter (Fig. 1).

Crypt depth. There was a 25% increase in the depths of the crypts during the first 28 days of postnatal life, from 148 to 199 μm ($r = 0.98$, $p < 0.01$) (Fig. 1).

C:V ratio. After an initial decline the C:V ratio increased by 25% from 6.7 at 14 days to 8.2 at 28 days ($p < 0.001$) (Fig. 2).

CCPR. There was no significant increase in CCPR until after day 14 when it increased by 50% from 3.9 cells/crypt/h to 5.6 cells/crypt/h ($p < 0.001$) (Fig. 2).

Table 1. *Number of animals, litters, and mean (SEM) body wt of guinea pigs studied on 1st to 28th postnatal days*

Postnatal day	1	7	14	21	28
No. of animals	17	13	19	15	15
No. of litters	4	4	6	5	5
Body wt (g)	88 (2)	122 (5)	177 (6)	224 (5)	307 (7)

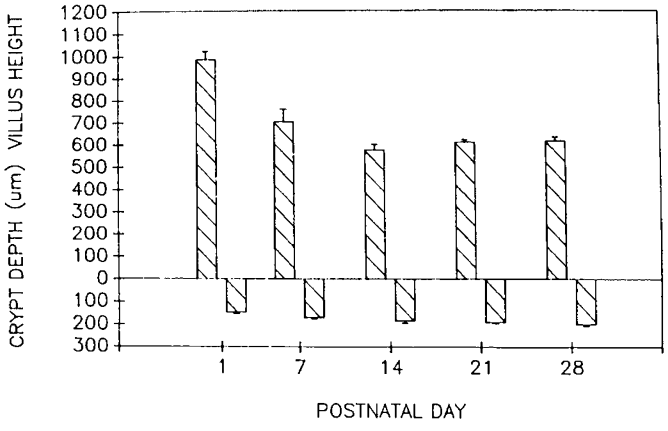

Fig. 1. Villus heights and crypt depths of upper intestinal mucosa of guinea pigs aged 1 to 28 days. Means and SEM are shown.

Net villus influx. This was calculated from the product of the C:V ratio and CCPR. An 80% increase in the net villus influx was seen during the first 28 days of life from 26.1 cells/villus/h to 45.9 cells/villus/h. The maximal rate of change occurred between the 2nd and 3rd postnatal wk ($p < 0.001$) (Fig. 3).

DISCUSSION

We have described the changes in upper intestinal epithelial architecture and kinetics of the guinea pig during the suckling and weaning periods. After an initial decline in villus height we have shown a proliferative change which was most marked during the 3rd postnatal wk. This was accounted for by an increase in both the depth and number of the crypts, and in CCPR, leading to an increase in the number of new cells entering the villus compartment. The absence of an increase in villus height indicates a concomitant increase in net villus eflux. These findings are in accord with those described in the mouse at weaning, in mechanism but not in timing (4).

The decline in villus heights and increase in crypt depths and CCPR is the continuation of a trend observed during the 1st postnatal wk (7, 13). During the immediate neonatal period these changes in mucosal architecture are accompanied by an increase in the length and number of microvilli on villus enterocytes (8), a decline in their glycogen content (14), and a cessation of their capacity to internalise macromolecules (15). The latter appears to occur independently of the rate of epithelial cell replacement (16), and may be due to an exhaustion of membrane available for endocytosis (17). During the late suckling period there is also

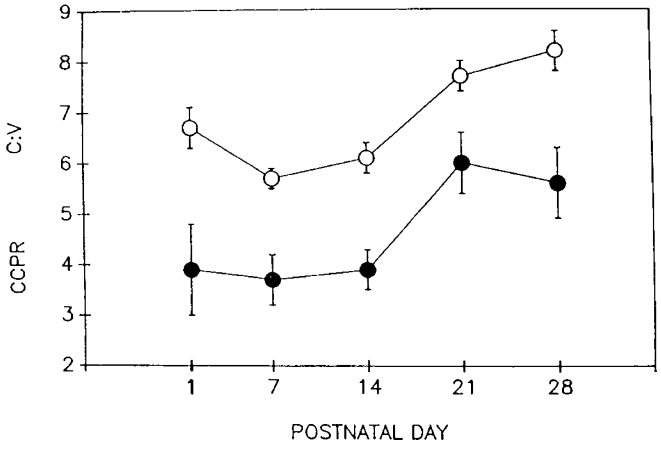

Fig. 2. C:V (O) and CCPR (●, cells/crypt/h) of guinea pigs. Mean values and SEM for animals aged 1 to 28 days are shown.

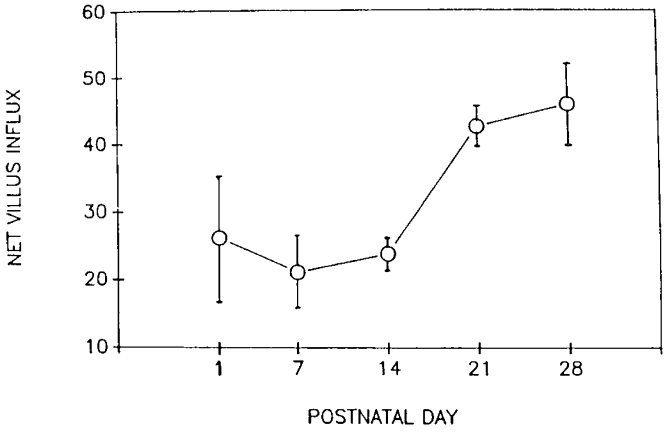

Fig. 3. Mean net villus influx (cells/villus/h) of guinea pigs aged 1 to 28 days. Error bars are SEM.

a gradual decline in mucosal lactase activity, and a slow rise in mucosal sucrase and maltase activities (9, 18).

This is followed by a second phase of postnatal intestinal adaptation: weaning, the process of transition from milk to solid diet. The introduction of solid diet and the decline in breast feeding occurs in concert with further alterations in intestinal mucosal morphology and function to accomodate these dietary changes.

In the precocial guinea pig vegetable diet may be consumed from soon after birth, and there is a gradual decline in breast feeding from around the 8th day reaching completion at around 2 wk of age (10). This process is reflected in the gradual changes in epithelial morphology and cytokinetics described here, and in mucosal disaccharidase activities (9, 18). The decline of lactase activity, and the achievement of adult levels of sucrase and maltase activity are not reached until around the 40th postnatal day (9).

Such gradual changes in mucosal morphology, function, and cytokinetics in the precocial guinea pig, accompany a relatively long and gradual weaning process. They contrast with the short and defined period seen in the rat. In this altricial species there is a constellation of changes in epithelial structure, function, and kinetics that occur between the 14th and 28th postnatal day in association with a relatively abrupt introduction of solids and termination of breast feeding (2).

It has been shown that these weaning changes are mediated by dietary, hormonal, and genetic factors (2, 5, 19). The timing of expression of lactase activity, for instance, appears to be determined by luminal substrate (20, 21), whereas that of sucrase activity is "hard-wired" (22, 23). Whatever the factors governing such weaning changes (local or central, substrate-induced or inherent), the mechanisms entail changes in epithelial cell structure and function. These must involve either changes in the epithelial cell population (24) and/or in intracellular processes such as transcription or translation of enzyme activity (2, 22, 25).

In the growing guinea pig not only are such weaning changes in mucosal disaccharidases very gradual, but are associated with morphologic and cytokinetic changes that occur largely during and after the termination of weaning in the 3rd and 4th wk. It seems likely, therefore, that in this precocial species in which solids may be consumed from early neonatal life, and in which breast feeding has virtually ceased by 2 wk of age, that luminal dietary substrates are not the major signal for the changes described. We suggest that the accelerated epithelial cell turnover seen during the 3rd and 4th wk is part of a developmental program of gut ontogeny that occurs independently of dietary influences. It is quite possible, however, that these cytokinetic changes are responsible for the continued rise in sucrase activity which does not reach adult levels until the 6th postnatal wk (9), and are, as in the rat, hardwired in expression (19).

These findings are in keeping with the hypothesis that birth is a "movable" event which occurs at different time points in the course of the ontogeny of the gut in different mammals (1, 26). In altricial species, such as the rat and mouse, birth occurs comparatively early in gut ontogeny, and weaning represents a major event in gastrointestinal maturation. In the precocial guinea pig, capable of consuming solid diet soon after delivery, birth occurs later in gut ontogeny. In this species weaning does not represent such a significant period in gastrointestinal development, and it appears that transition from milk to solid diet is not the major trigger for the changes that we have described.

It remains to be seen what factors control the postnatal development of the gastrointestinal tract of the precocial guinea pig, in particular whether the neonatal rise and fall in mucosal lactase activity occurring independently of major cytokinetic changes, is hardwired or governed by dietary factors. The guinea pig, which in many respects more closely resembles man than the rat and mouse, may represent a more suitable model for our understanding of human gastrointestinal maturation.

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