Enterocyte Dynamics and Mucosal Development in the Posthatch Chick

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ABSTRACT Changes in the morphology of the small intestinal mucosa and enterocyte dynamics were examined in posthatch chicks through 12 d. At hatch, enterocytes were round and nonpolar; however, within 24 h posthatch, enterocytes lengthened and exhibited more typical morphology. Crypts were rudimentary at hatch and by 48 h invagination was completed and crypt numbers increased by branching and fission, with the number of crypts per villus reaching plateau after 72 h posthatch. All epithelial cells were proliferative at hatch. In the crypts, the proportion of proliferating enterocytes decreased to 50 to 60% within 2 d posthatch, whereas along the villus the proportion of proliferating cells decreased to 10 to 20% by 6 d.

Different patterns of temporal development of villi were observed in the duodenum, jejunum and ileum. Individual villus surface area increased steadily in the duodenum throughout the experiment, whereas individual jejunal and ileal villus surface areas increased more slowly after 4 d posthatch. The number of villi per cross-section of intestine increased in the duodenum and jejunum but not in the ileum. The total segment villus surface area increased similarly in all segments until 3 d posthatch, after which the jejunum increased considerably in absorptive area, whereas the duodenum and ileum increased more slowly. This study shows that, in the hatching chick, the small intestine matures in a manner similar to neonatal mammals, with specific ontogenetic timetables in the different small intestinal segments, however, the most dramatic changes occur within the first 24 h posthatch.

(*Key words*: chick, ontogeny, small intestine)

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INTRODUCTION

As the marketing age of broilers decreases, the length of the transition from endogenous yolk dependence to exogenous food utilization becomes increasingly critical. The development of the supply organs must be such that sufficient precursors for growth are available.

Intestinal development in chicks differs from that of mammals, and dramatic changes in villus size and volume have been described after hatching (Overton and Shoup, 1964; Uni et al., 1995a,b, 1998). The present day broiler is selected for rapid growth, which has considerably influenced the rate of intestinal development (Smith et al., 1990). A further difference from mammals is that in the chick jejunum, proliferation of enterocytes is not confined to the crypt but also occurs along the villus (Uni et al., 1998).

Intestinal development in mammals has been extensively examined. The timing and extent of intestinal development in different mammalian species often reflects the length of the gestational period. In the human fetus at 20 weeks gestation, intestinal morphology and many functional parameters resemble those of the newborn (Lebenthal, 1989). In altricial species such as the rat, villi are not formed until just prior to birth, and several aspects of functional development are initiated in the postnatal period (Montgomery et al., 1981; Henning, 1987). Intestinal ontogenesis during the suckling and weaning periods involves extensive cryptogenesis and epithelial proliferation. At the same time, major changes occur in gene expression for microvillar and intracellular enzymes, transport proteins, and receptors for circulating and luminal trophic factors and hormones (Perozzi et al., 1993).

This study provides new information concerning cryptogenesis, villus development, and enterocyte dynamics in the small intestines of the posthatch broiler chick.

MATERIALS AND METHODS

Experimental Procedures

Male broiler chicks (Ross; n = 120) were obtained from a commercial hatchery[2] within 1 h of clearing the shell

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Abbreviation Key: BrdU = 5-bromo-2-deoxyuridine; PCNA = proliferating cell nuclear antigen.

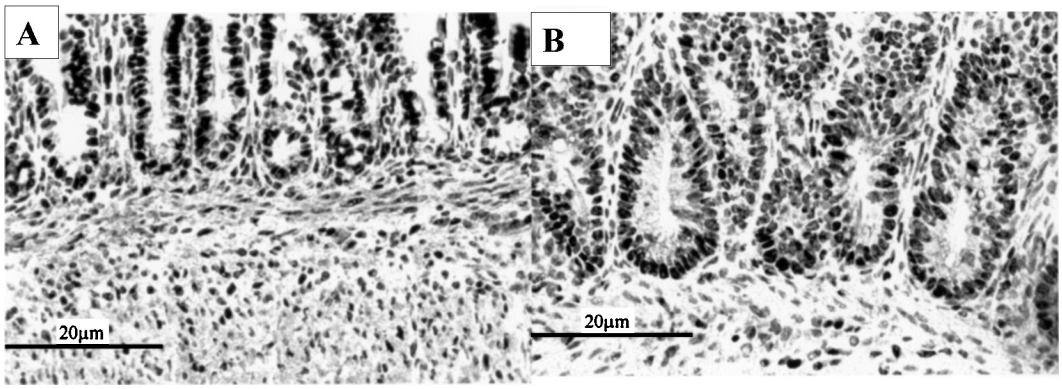

FIGURE 1. Crypts at different stages of development. Light microscopy of jejunal sections: (A) 2 h posthatch; (B) 96 h posthatch.

and were transported within 30 min to a temperature-controlled facility. Upon arrival, chicks were weighed and had free access to water and a commerical diet formulated to meet or exceed NRC requirements (National Research Council, 1994) for the entire experimental period (12 d). All procedures were approved by the Animal Care and Ethics Committee of our institute.

5-Bromo-2-deoxyuridine Labeling

An aqueous solution[3] containing 5-bromo-2-deoxyuridine (BrdU) and 5-fluoro-2-deoxyuridine (10:1, vol:vol) was injected intraperitoneally at 1 mL/100 g body weight. Injections were made at five ages posthatch: 0, 24, 96, 168, and 240 h. At 2, 12, 24, 48, 72 and 96 h postinjection, four birds were killed, and small intestines were sampled.

Histology

Segments of approximately 2 cm were taken from the midpoint of the duodenum (duodenum), from the midpoint between the point of bile duct entry and Meckel's diverticulum (jejunum), and midway between Meckel's diverticulum and the ileo-ceacal junction (ileum). Segments were gently flushed twice with 0.9% NaCl to remove the intestinal contents and were fixed in fresh 4% buffered formaldehyde. All samples were dehydrated, cleared, and embedded in paraffin. Sections were cut at 5 μm and were placed on glass slides; for crypt examination, serial sections were taken. For all assays, sections were deparaffinized in xylene and rehydrated in a graded alcohol series. For immunohistochemistry, sections were incubated in 3% hydrogen peroxide in methanol for 10 min to quench endogenous peroxidase. Proliferating cell nuclear antigen (PCNA)-positive cells were measured by use of monoclonal anti-PCNA antibody followed by the use of peroxidase-ABC (Zymed PCNA staining kit)[3] according to the manufacturer's directions. Incorporation of BrdU was indicated by monoclonal anti-BrdU antibody followed by the use of peroxidase-ABC according to the manufacturer's directions.[3] Counterstaining was with hematoxylin; samples were dehydrated and mounted in Histomount.[3] Negative control slides, without antibodies, were prepared in all experiments.

Measurements

Cell proliferation was examined using PCNA and BrdU labeling, and cell migration was examined using BrdU labeling. Crypts were defined as the invaginations containing mitotic activity at the base of villi. Cell length was the distance from brush-border membrane to the basolateral membrane. Villus surface area was calculated from villus height and width at half height. The total villus segment surface area was calculated by multiplying the individual villus surface area by the number of villi per intestinal cross-section and by the length of the intestinal segment. Migration rate was calculated from the time of BrdU injection until the time that cells reached a defined point on the villus divided by the distance from the nearest crypt.

Statistical Analysis

Data were analyzed by analysis of variance using the general linear models procedures of SAS software (1986). Significant differences between treatment means were examined by *t*-tests. Significance was at 5% unless otherwise stated.

RESULTS

Crypt Formation

At hatch, crypts contained few cells, and invagination was not complete throughout the small intestine. Rapid changes occurred posthatch, and by 48 h, invagination was complete in all segments (Figure 1). The process of crypt enlargement and fission continued throughout the experiment. The number of crypts per villus increased intensively during the first 48 h posthatch in all segments

[3]Zymed Laboratories, San Francisco, CA.

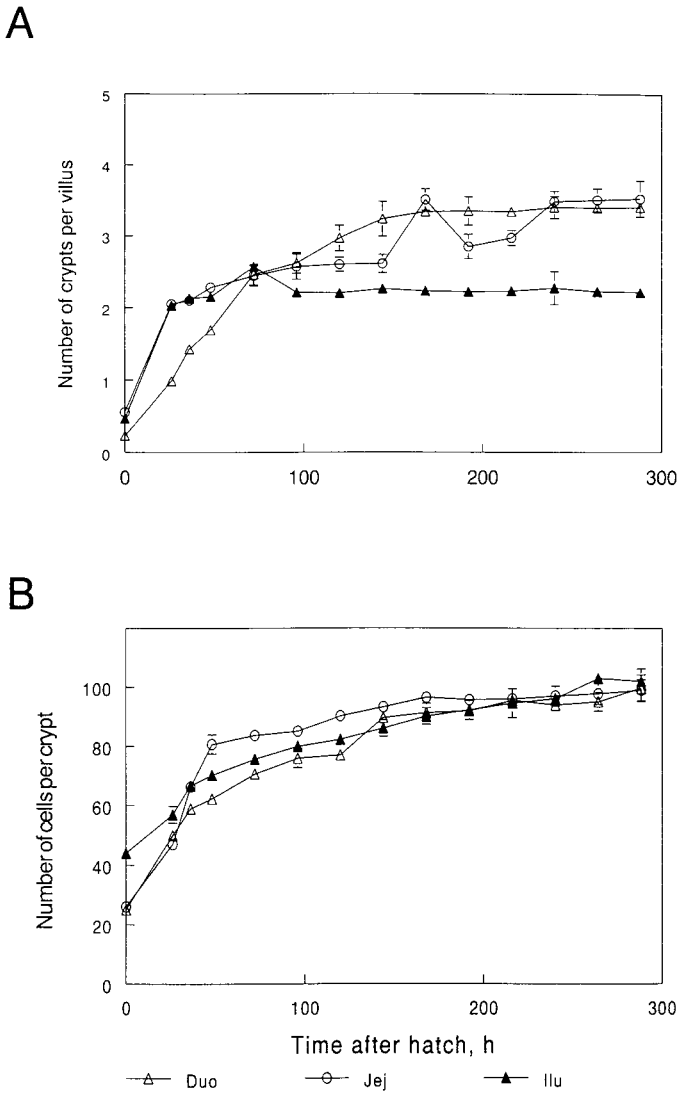

FIGURE 2. Changes in some characteristics of crypts with age in posthatch chicks. (A) number of crypts per villus; (B) number of cells per crypt cross section. Points are means, and bars are SE and are shown when they do not fall within the symbol. In Panel A, the ileum was significantly lower than the other segments after 96 h. Duo = duodenum; jej = jejunum; ilu = ileum.

(Figure 2A). In the duodenum and jejunum, there was further gradual increase until 216 h posthatch. The number of crypts per villus reached a plateau in the ileum after 72 h posthatch.

The number of cells in a crypt cross-section was higher at hatch in the ileum than in the more proximal intestinal segments. Cell numbers per crypt increased rapidly in all small intestinal segments until 72 h, after which the changes became small (Figure 2B).

Proportion of PCNA-Positive Cells in the Crypt

At hatch, almost all cells were proliferating; however, the proportion of PCNA-positive cells in the crypt decreased rapidly with time in all three intestinal segments, reaching about 50% of proliferating cells after 72 h. After this period, the proportion of PCNA-positive cells in the crypt was stable with no significant difference between the segments (Figure 3).

Proportion of PCNA-Positive Cells in the Villus

At hatch, almost all cells along the villus were PCNA positive in all intestinal segments. However, with age, the percentage of PCNA-positive cells along the villus decreased at differing rates in the three intestinal segments (Figure 3). At 72 h posthatch, the proportion of PCNA-positive cells ranged from 10 to 40%. At 168 h, this proportion decreased to 10 to 20%; after that time the proportion remained stable. The slowest decrease in proliferating cells was observed in the jejunum.

Cell Size and Enterocyte Morphology

At hatch, in all intestinal segments, the majority of enterocytes were relatively nonpolar and lacked a clearly

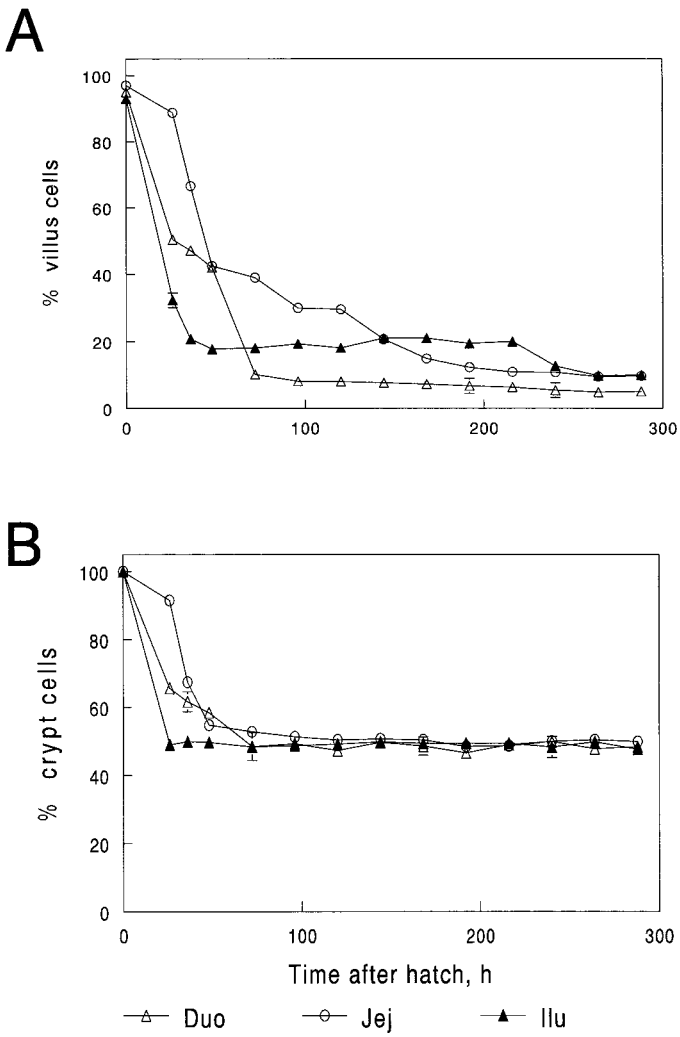

FIGURE 3. Proportion of proliferating cell nuclear antigen-positive cells with age along the villus (A) and in crypts (B) in posthatch chicks. Points are means, and bars are SE and are shown when they do not fall within the symbol. Duo = duodenum; jej = jejunum; ilu = ileum.

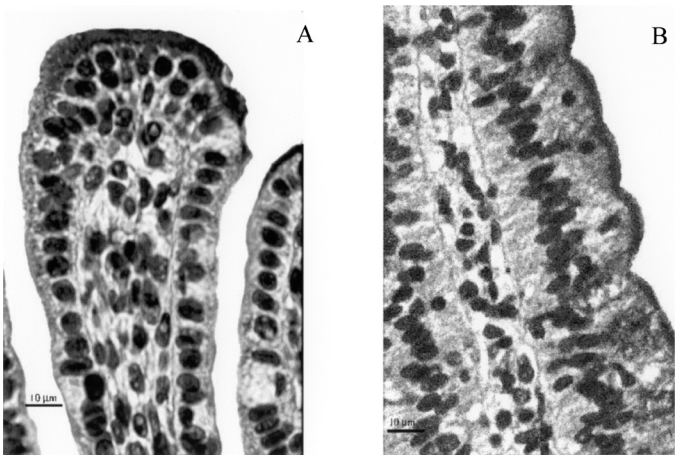

FIGURE 4. Typical villus enterocytes in chicks at (A) 2 h posthatch and (B) at 168 h posthatch.

visible brush-border membrane. However, these cells gained polarity and increased in length, and a distinct brush border was apparent after the first 24 h posthatch (Figure 4). Enterocyte width changed less than 5% with age (data not shown) whereas the cell length increased considerably. At hatch enterocyte length was similar in all intestinal segments (Figure 5); however, in the 24 h posthatch cell, length in all intestinal segments increased and then plateaued. In the duodenum and jejunum a further increase in cell length was observed after 100 h, reaching a plateau in the duodenum after 216 h and in the jejunum after 144 h.

Villus Length and Width and Surface Area

At hatch there were significant differences in villus length between segments with duodenal villi longer than those in the jejunum and ileum (data not shown). At hatch the villus width was similar in all the intestinal segments and changed little, increasing slightly in the duodenum and ileum with age (data not shown). These data were used to calculate villus surface area (Figure 6A). In the duodenum, villus surface area increased most rapidly and continued to increase until 240 h. In contrast, jejunal and ileal villus surface area increased more slowly and tended to plateau after 168 h.

Villi per Cross Section

The highest number of villi per intestinal cross-section at hatch was found in the ileum, and this number changed little with time. In the duodenum and jejunum the number of villi per intestinal cross-section increased after hatch, reaching a plateau after 72 h in the duodenum and 216 h in the jejunum (Figure 6B).

Total Intestinal Segment Absorptive Area

The total surface area in the different intestinal segments was calculated from the surface area of each villus,

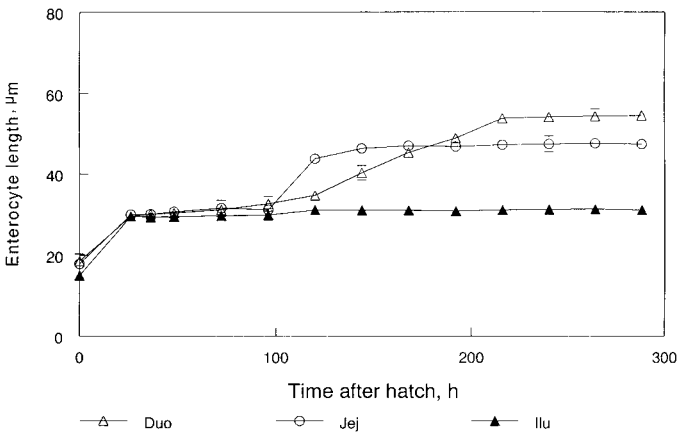

FIGURE 5. Enterocyte size with age in posthatch chicks. Points are means, and bars are SE and are shown when they do not fall within the symbol. Duo = duodenum; jej = jejunum; ilu = ileum.

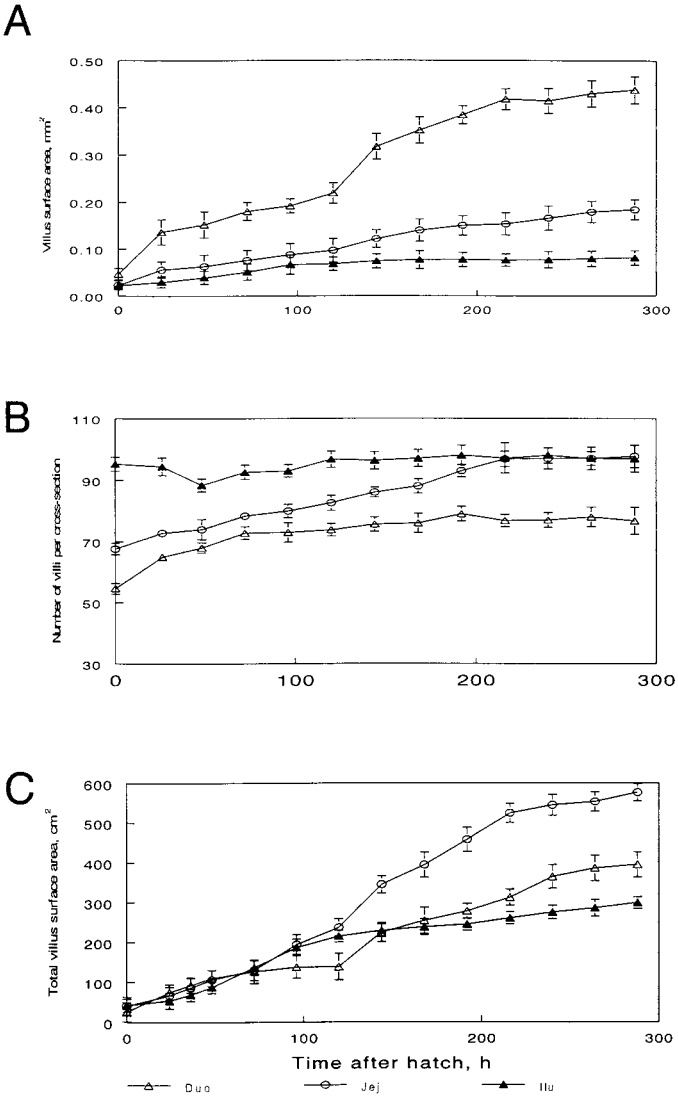

FIGURE 6. (A) Villus surface area, (B) number of villi per intestinal cross section, and (C) total segment surface area in posthatch chicks. Points are means, and bars are SE and are shown when they do not fall within the symbol. Duo = duodenum; jej = jejunum; ilu = ileum.

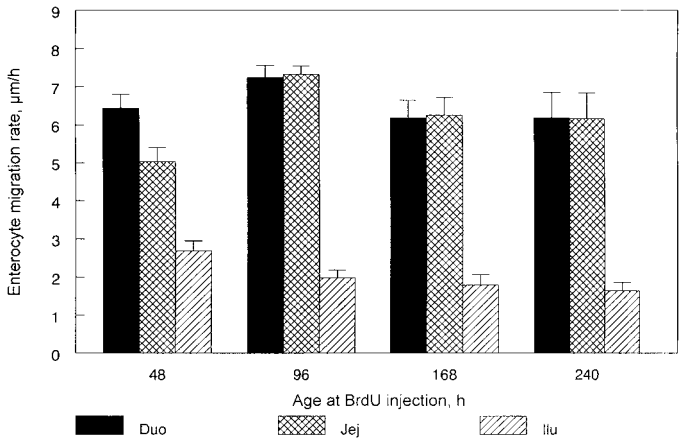

FIGURE 7. Migration rate of enterocytes in segments of the small intestines. The X-axis is the age in hours at the time of the 5-bromo-2-deoxyuridine (BrdU) injection. The results are means, and bars are SE.

the number of villi per cross-section, and the length of the segment (Figure 6C). At hatch the three small intestinal segments had similar total surface areas that increased in the same way with age until 72 h. However, after this time, jejunal surface area increased more than the other intestinal segments, reaching values more than twofold higher than the other intestinal segments.

Migration Rate

Enterocyte migration rate at different developmental stages was determined following BrdU injections. Because most of the villus cells were proliferative at hatch, we could not determine migration before 48 h posthatch. The migration rate of enterocytes in the ileum was lower than that of enterocytes in the duodenum and jejunum throughout the experiment (Figure 7). At 48 h posthatch the highest migration rate was observed in the duodenum; however, after this time, migration rate was similar in the duodenum and jejunum but lower in the ileum.

DISCUSSION

Striking changes in the morphology of the small intestinal mucosa in chicks occur close to and after hatching, including enterocyte maturation, intensive cryptogenesis, and villus growth. The morphological and immunhistochemical studies described in this paper have noted different temporal patterns of development in the three segments of the chick small intestine.

In the mature bird, the small intestinal epithelium is continuously renewed by proliferating crypt cells that migrate up the villi. These cells differentiate, and apical microvilli and absorptive functions develop during their movement toward the tip of the villi (Simon and Gordon, 1995). The mature polar enterocyte has a defined cellular structure that is probably required for optimal absorptive function. At hatch, chick enterocytes were small, round-shaped, and lacked a well-defined brush border; however, cells became increasingly polar after 24 h posthatch. Enterocyte differentiation involves the polarization and the stereotypic arrangement of the brush-border cytoskeleton (Fath et al., 1993). Immature enterocytes have also been reported postnatally in other species (Quaroni, 1985). In the mouse, morphogenesis is not complete until the third postnatal week (Calvert and Pothier, 1990), and in the pig, the ultrastructure of the microvillus surfaces of the villus enterocytes develops slowly in the postnatal period (Smith and Peacock, 1989).

From this study it appears that the ontogeny of chick enterocytes can be divided into two periods. In the first 24 h after hatch, the enterocytes acquired polarity and a distinct brush-border membrane. The second period involves hypertrophy, which was expressed mainly by increased cell length. In the duodenum and the jejunum the processes of differentiation and hypertrophy are separate; in the jejunum hypertrophy was extended from 72 to 144 h posthatch, whereas in the duodenum this process continued until 216 h posthatch. Little hypertrophy was observed in the ileum. Thus, morphologically, the enterocytes in the ileum may be relatively mature at hatch, whereas development of enterocytes in the duodenum and jejunum continued for several days posthatch.

Crypt development is a crucial step in intestinal maturation. In mammals the small intestinal crypt contains 200 to 300 cells per crypt (Loeffler et al., 1986). In the adult mouse, small intestine cell proliferation is restricted to the crypts, and cells become committed to a particular differentiation pathway in the upper third of the crypts (Cheng and Leblond, 1974). In rats and mice, crypts develop during the early postnatal period from the flat intervillus epithelium (Mathan et al., 1976; Calvert and Pothier, 1990). In this study, crypts in hatching chicks were not fully defined, especially in the jejunum, and over the first 24 h posthatch the crypts developed into distinct structures. At hatch, all crypt cells were proliferative, and this proliferative activity is probably required for crypt enlargment and fission. The increase in the number and size of crypts has two direct effects: 1) providing enterocytes for the increasing intestinal absorptive surface area as the villi grow and 2) increasing cell renewal rate. A similar process has been reported in the mouse. During the third postnatal week in the mouse, the rate of epithelial cell production increases as crypts multiply by fission (Cheng and Bjerkens, 1985). The number of the proliferative crypts that support each villus then directly influences the magnitude and intensity of villi growth.

In this study, as in previous reports (Uni et al., 1998), enterocytes along the villus were shown to proliferate as indicated by PCNA and BrdU labeling. In the rat, Hermos et al. (1971) have shown that there is no well-defined proliferative region in the small intestine of fetal rats at Day 21 of gestation; DNA synthesis and cell proliferation occur in epithelial cells over the entire length of the villi. In this study in the majority of villus cells in hatching chicks were mitotic. However, this pattern changed with the number of proliferating cells along the villus, decreasing with age. Cell mitosis along the villus may well play an important role in posthatch hyperplasia. Enlargement

of crypts and crypt branching might have compensated for the reduction of mitotic cells along the villi as growth occurred.

The extent of the decrease in the proportion of proliferating cells along the villus was characteristic for each segment, with the slowest decline in the jejunum. The differences in villus proliferation in the different segments would suggest that villus growth in the jejunum is largely due to villus mitosis, which would be greater than that in the duodenum where villus proliferation is smaller.

Because at hatch almost all villus cells in all segments were proliferative, determination of migration rate by following cells that were BrdU labeled was not possible. However, once the rate of proliferating cells in the villi decreased, migration rate could be measured. Thus, migration rate changed little after 72 h posthatch, and ileal enterocytes migrated more slowly than duodenal and jejunal enterocytes through 288 h posthatch. In the rat, cell migration in the suckling period is slower than that in the mature period, although this is not true for the pig or guinea pig (Grand et al., 1976).

Previous studies of birds have detailed changes in villus size and surface area close to hatch (Uni et al., 1995a,b, 1998). In this study, we have presented villus surface area and the number of villi in an intestinal cross-section. We have used these data with segment length to calculate the total absorptive area in each intestinal location. From these determinations, it appears that surface area was similar in all segments at hatch. With time, however, growth in the jejunum resulted in the greatest absorptive area that began to plateau after 240 h posthatch. Duodenal and ileal surface area changed differently with time, although both increased little after 240 h.

The absorptive capability of the intestine would be expected to be correlated with the brush-border membrane surface area and the number of carriers and transporters available. This absorptive capacity would, thus, be represented by the total surface area of the intestinal segment.

Previous in vivo studies with nonabsorbed markers have indicated that major absorption occurs in the duodenum and proximal jejunum with decreasing uptake distally (Noy and Sklan, 1995). This uptake is not parallel to the pattern of the total intestinal surface area in the different segments. Surface area, however, would represent absorptive potential, whereas actual uptake is dependent on substrate concentration and availability and carrier and transporter concentrations and turnover rates.

There has been speculation as to whether the absorptive capacity of the intestine is a rate-limiting step in growth. Obst and Diamond (1992) have suggested that chicks have only a small capacity for excess absorption relative to intake, whereas other studies have indicated a large capacity for excess intestinal uptake. These studies have suggested that feed intake may be the limiting process (Noy and Sklan, 1996). This study suggests that intestinal surface area does not appear to be limiting absorption in the young chick.

In the hatching chick the small intestine matures largely in the immediate posthatch days, which occurs in a manner similar to neonatal mammals, with intensive cryptogenesis, enterocyte maturation to functional forms, and localization of proliferation to the crypts.

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