Morphological, Molecular, and Functional Changes in the Chicken Small Intestine of the Late-Term Embryo

Z. Uni,[1] E. Tako, O. Gal-Garber, and D. Sklan

The Faculty of Agricultural, Food and Environmental Quality Sciences, Department of Animal Sciences, P.O. Box 12, Rehovot 76100, Israel

ABSTRACT The rapid development of the gastrointestinal tract posthatch has been described; however, little information exists concerning the development of the small intestine in the prehatch period. The present study examined the morphological, cellular, and molecular changes occurring in the small intestine toward the end of the incubation period by examining the expression of intestinal genes that code for brush border digestive enzymes and transporters, their biochemical activities, and the morphological changes in the mucosal layer. The results indicated that during the last 3 d of incubation the weight of the intestine, as a proportion of embryo weight, increased from approximately 1% on d 17 of embryonic age to 3.5% at hatch. At this time the villi could be divided into two main developmental stages, differing in their length and shape, with the larger villi often being pear-shaped and the smaller villi being narrower and having a rocket-like shape. However, on d 19 a further stage of villus development was observed. Activities of maltase, aminopeptidase, sodium-glucose transporter (SGLT)-1, and ATPase began to increase on d 19 and further increased on the day of hatch. The expression of mRNA for these brush-border membrane (BBM) enzymes and transporters was detected from d 15. Determining quantities relative to β-actin indicated that expression of all parameters examined was low on d 15 and 17, increased 9- to 25-fold on d 19, and all decreased again on the day of hatch. Relative expression of mRNA of the different enzymes and transporters were correlated as were their activities (r = 0.75 to 0.96); however, expression was not correlated with enzymatic activities. The role of these parameters in the ontogeny of absorption is discussed. Thus, major changes in the expression and localization of the functional brush-border proteins prepare the framework for ingestion of carbohydrate- and protein-rich exogenous feed posthatch.

(*Key words*: embryo, intestine, brush border enzyme, functional change)

2003 Poultry Science 82:1747–1754

INTRODUCTION

In the hatching chick, yolk provides nutrient reserves for several days; however, chicks are precocial and directly forage for exogenous feed. The immediate posthatch period is characterized by a transition from the use of lipid-rich yolk as the nutrient source to exogenous feed rich in carbohydrates and proteins (Sklan, 2001). This transition is accompanied by rapid physical and functional development of the gastrointestinal tract (Uni et al., 1999). During the first week posthatch the small intestines increase in weight more quickly than the body mass (Katanbaf et al., 1988; Sell et al., 1991; Sklan, 2001), and rapid morphological changes occur after hatch with differing ontogenic timetables of villus growth in the duodenum, jejunum, and ileum (Sklan, 2001). The accelerated rate of development posthatch is reflected in the several-fold elevation in numbers of enterocytes during first few days posthatch, resulting from the dramatic increase in villus length (Geyra et al., 2001). Enterocytes, which are round and nonpolar at day of hatch, increase rapidly in length and develop pronounced polarity and defined brush border within hours posthatch (Geyra et al., 2001). Crypts begin to form on the day of hatching and become defined within 2 to 3 d, increasing both in cell numbers and in size while branching is initiated (Uni et al., 2000; Geyra et al., 2001). In parallel with these morphological changes, the ability of the intestinal tissue to digest and absorb nutrients increased steadily during first week posthatch (Uni et al., 1999; Sklan, 2001). Pancreatic enzyme activity was observed in the small intestines prehatch (Marchaim and Kulka, 1967) and increased posthatch (Sklan and Noy, 2000). Furthermore, brush-border enzyme activities also increased rapidly in the posthatch days (Uni et al., 1999).

Received for publication February 2, 2003.
Accepted for publication June 9, 2003.
[1]To whom correspondence should be addressed: uni@agri.huji.ac.il.

Abbreviation Key: AP = aminopeptidase; ATPase = Na^+K^+-ATPase; BBM = brush-border membrane; SI = sucrase-isomaltase; SGLT = sodium-glucose transporter.

Although the rapid development of the gastrointestinal tract posthatch has been described, little information exists concerning the development of the small intestine in the prehatch period. Studies have been carried out on morphological development and alkaline phosphatase and maltase activities of the intestinal epithelium in culture (Black, 1978; Black and Moog 1978). The present study examined morphological, cellular, and molecular changes occurring in the small intestine toward the end of incubation. Intestinal development was assayed by examining expression of intestinal genes that code for brush-border digestive enzymes, their transporters, and their biochemical activities and by the morphological changes in the mucosal layer.

MATERIALS AND METHODS

Embryonic Tissue Sampling

Embryos (Ross × Ross) were obtained from a commercial hatchery[2] from a maternal flock 46 wk in lay. Twenty embryos were killed for analysis at 15, 17, 19, 20 d of incubation (15E, 17E, 19E, 20E, respectively) and on the day of hatch (within 2 h of clearing the shell). For each embryo, body (with yolk sac) and small intestine weights were recorded. The jejunum was removed as previously described (Uni et al., 1995, 1998), and segments (1 cm long) were taken and placed in three separate tubes: 1) frozen in liquid nitrogen and stored at –80°C for RNA expression analysis, 2) fixed in 4% neutral-buffered formalin solution for histology, and 3) stored at –20°C for determination of brush-border membrane (BBM) and transporter activities.

Morphological Examination

Intestinal samples from 15E, 17E, 19E, 20E, and day of hatch were dehydrated, cleared, and embedded in paraffin. Serial sections were cut at 5 μm and placed on glass slides. Sections were deparaffinized in xylene, rehydrated in a graded alcohol series, and examined by light microscopy (Uni et al., 1995, 1998). Morphometric indices were determined by computer analysis[3,4].

RNA Isolation, Probe Preparation, and Northern Hybridization

Briefly, total RNA was isolated from chicken jejunal tissue using TRI-REAGENT-RNA/DNA/protein isolation reagent[5] (1 mL/100 mg of tissue) according to the manufacturer's protocol. The integrity of the RNA was verified by ethidium bromide staining, and the RNA concentration was determined spectrophotometrically. For Northern blot analysis, 30 μg of total RNA from the jejunum was denatured and separated by electrophoresis in a 1.5% agarose/1.1 *M* formaldehyde gel. After electrophoresis, RNA was transferred overnight by capillary transfer to a nylon filter (Hybond-N[6]) and then fixed on the filter by exposure to ultraviolet light at 340 nm for 2 min.

Four probes were used for hybridization: 1) cDNA fragment from the chicken intestine aminopeptidase gene (Gal-Garber and Uni, 2000), 2) cDNA fragment from the chicken intestine sucrase isomaltase gene (Uni, 1998), 3) cDNA fragment from the chicken sodium-glucose transporter gene (Gal-Garber et al., 2000), and 4) cDNA fragment from the chicken Na^+K^+ATPase gene (Gal-Garber et al., 2003).

The amount of total RNA per lane was determined through rehybridization with a probe for the constitutively expressed transcript for chicken β-actin. The five probes were labeled[7] with ^{32}P-dCTP by the random prime labeling method After a high-stringency wash (0.1× SSC/0.1% SDS at 57°C), blots were exposed for 24 h at –70°C to x-ray film in the presence of an intensifying screen. The abundance of sucrase-isomaltase (SI), aminopeptidase (AP), sodium-glucose transporter (SGLT)-1, and Na^+K^+ATPase transcripts was normalized to the density of β-actin transcripts by densitometer scanning.

Brush-Border Enzymes and Transporter Activities

Enzyme activities were assayed in homogenized jejunal tissue (250 mg tissue/5 mL of 50 m*M* sodium phosphate buffer, pH 7.2). Maltase (EC 3.2.1.20) activity was assayed colorimetrically using maltose as a substrate (Dahlquist, 1964; Palo et al., 1995) and expressed as millimoles of glucose released per minute per gram of jejunal protein. AP activity (EC 3.4.11.2) was determined by hydrolysis of L-leucine-p-nitroanilide for 15 min at 37°C, p-nitroanilide was determined spectrophotometrically at 405 nm according to Benajiba and Maroux (1980), and AP activity was defined as the production of 1 μmol of p-nitroanilide per minute per gram of jejunal protein.

The SGLT-1 uptake activity was determined at 37°C. Briefly, 10 μL of the homogenate was added to 190 μL of solution containing either 150 m*M* NaCl or KCl and 30 μM D-glucose-[6-^{3}H(n)]. The reaction was stopped after 3 s by addition of 2 mL of an ice-cold solution containing 150 mmol/L NaCl and 0.25 mmol/L phlorizin (Lescale-Matys et al., 1993).

The Na^+-K^+-ATPase kinetic measurements were conducted and phosphate was measured by spectrophotometry at 690 nm using a kit (catalog no. 670).[8] Na^+-K^+-ATPase activity was expressed as millimoles of Pi per gram of protein per min and determined as the difference between total ATPase activity and ouabain-insensitive ATPase activity (Gal-Garber et al., 2003). Total protein was

[2]Kvuzat Yavne, Yavne, Israel.
[3]Photoshop 4.0, Adobe System Incorporation, CA.
[4]NIH Image, Bethesda, MD.
[5]Tri-Reagent Molecular Research Center, Inc., Cincinnati, OH.
[6]Amersham Pharmacia Biotech, Amersham, U.K.
[7]Biological Industries, Kibbutz Beit-Haemek, Israel.
[8]Sigma Diagnostics, Sigma, St. Louis, MO.

TABLE 1. Weight and relative weight of the small intestine from 17 d of incubation (17E) until the day of hatch

Item	17E	19E	20E	Hatch
Embryo BW (g)	32.84 ± 0.81[a]	33.93 ± 0.93[a]	36.48 ± 1.46[a]	44.59 ± 0.06[b]
Intestine weight (g)	0.46 ± 0.05[a]	0.58 ± 0.05[b]	0.85 ± 0.047[c]	1.54 ± 0.06[b]
Intestine with BW (%)	1.4%	1.7%	2.3%	3.4%

[a–c]Values (n = 20) in rows not followed by the same superscript differ significantly ($P < 0.05$).

determined using the Bio-Rad protein assay[9] for protein concentration following detergent solubilization.

Statistical Analysis

Results were analyzed by ANOVA and by linear regression using the general linear models procedure of SAS software (SAS, 1986). Differences between ages were compared by the Tukey test following ANOVA, and values were considered statistically different at $P < 0.05$. Results are reported as least squares means with standard errors. Linear regressions were examined between mRNA expression of the different transporters and enzymes, between different enzyme activities, and between mRNA expression and the enzyme activities.

RESULTS

As incubation progressed BW of the embryos increased, as did the weight of the small intestines. However, small intestinal weight increased at a much greater rate than BW, which showed little increase close to hatching. The enhanced growth rate of the small intestine is clearly shown by presenting the weights of the intestine as a proportion of the embryo weight (Table 1). During the last 3 d of incubation, this ratio increased from approximately 1% on 17E to 3.5% at hatch.

The morphology of the small intestine also changed rapidly. Histology indicated that the intestine including the external muscular layers and the villi were growing rapidly. Villi at 15 d were rudimentary; however, on d 17 villi at different stages of development were observed (Figure 1). At this time the villi could be divided into two main developmental stages (V1, V2), differing in length and shape (Table 2), with the larger villi often being pear-shaped and the smaller villi (approximately 65% of the size of the larger villi) being narrower and with a rocket-like shape. Adjacent to each larger villus there appeared to be one smaller villus. A similar pattern was observed on d 18 and 19, with villi at both developmental stages growing, mainly in length, while maintaining similar size and distribution ratios. Examination of the base of the villi on d 19 indicated some budding at the base of existing villi. These buds developed considerably, and by d 20 an additional wave of small villi composed some 30% of total villi (Figure 2b). The earlier waves of villi had contin-

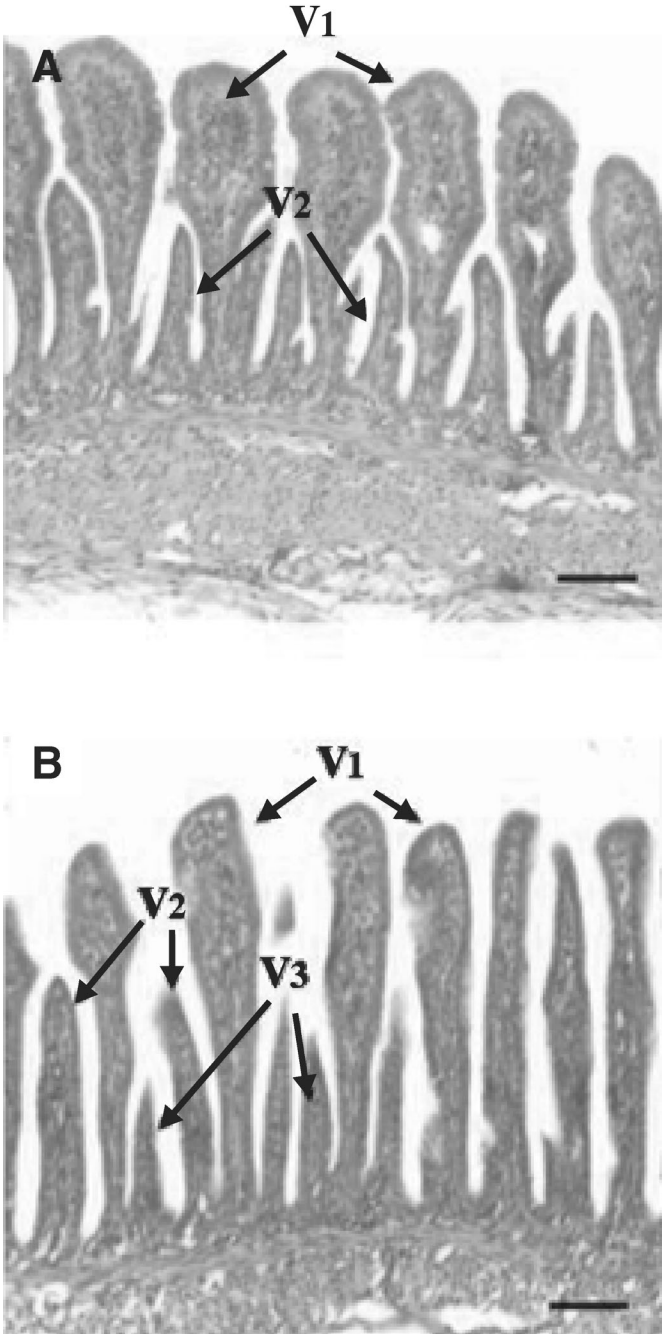

FIGURE 1. Representative light microscopy (×200) of intestinal villi from the jejunum of the broiler embryo at 17 d of incubation (A) and at 20 d of incubation (B). Sections show the pear-shaped villi and rocket-shaped villi. V1 to V3 = villi in different stages of development. Staining was with hematoxylin-eosin. Bar = 10 μm.

[9]Bio-Rad Laboratories, Hercules, CA.

TABLE 2. Morphological measurements of embryonic jejunual villi, in different stages of development, from d 17 of incubation (17E) until hatch

	Mean villus height (μm)			
	17E	19E	20E	Hatch
Villi stage 1 (V1)	180.2 ± 4.2[a] (53%)	186.8 ± 4.9[a] (55%)	250.9 ± 10.9[a] (38%)	553.7 ± 5.8[a] (41%)
Villi stage 2 (V2)	103.9 ± 3.9[b] (47%)	121.4 ± 3.9[b] (46%)	170.7 ± 11.6[b] (33%)	350.8 ± 11.3[b] (32%)
Villi stage 3 (V3)	—	—	107.2 ± 10.2[c] (29%)	142.11 ± 10.2[c] (27%)

[a–c]Values (n = 20) in the same columns not followed by the same superscript differ significantly ($P < 0.05$). Numbers in parentheses denote the percentage of villi at this stage.

ued to grow, and the third series of developing villi were now approximately 65% of the size of the next largest villi. The rate of growth of V1 and V2 between 19E and 20E was 30 to 40%; however, between d 20 and hatch, growth was twofold in the larger villi and somewhat less in the smaller V3 villi. The distribution of the three villus stages did not change between 20 d and hatch. Further rapid changes were observed posthatch; however, these have been previously described (Sklan, 2001).

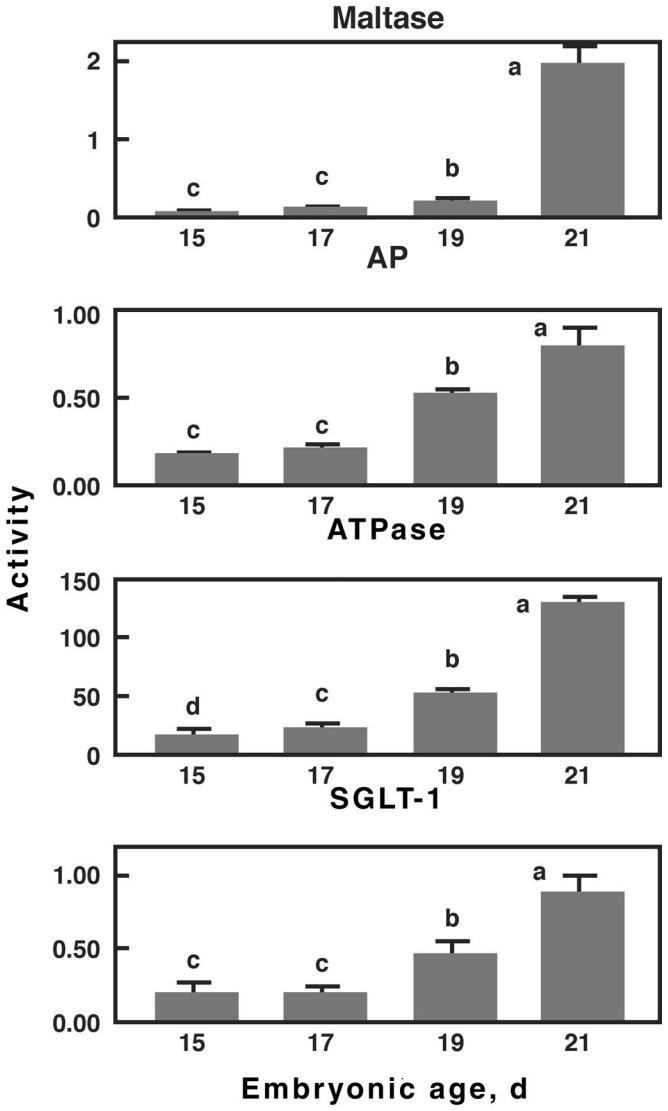

FIGURE 2. Activity of the brush-border enzymes sucrase-isomaltase (A), aminopeptidase (AP) and the transporters (B), Na+K+ATPase (C), and sodium-glucose transporter-1 (SGLT-1) (D) in the embryonic small intestine at 15, 17, 19 d of incubation and on the day of hatch. [a–d]Columns with different letters differ significantly ($P < 0.05$). Results are means, and bars are SE from 20 embryos.

Examining the activities of the jejunal BBM enzymes during the last period of embryonic development (Figure 2) indicated low activity of sucrase (not shown), maltase, and AP on d 15 and 17. Activities of all the enzymes and transporters examined began to increase on d 19 and further increased on the day of hatch. Activities of all enzymes were correlated (r = 0.73 to 0.92).

Expression of mRNA for these BBM enzymes and transporters was determined at the same intervals (Figures 3 and 4) in the embryonic jejunum. Expression of SI, AP, and SGLT-1 genes were detected from d 15. Determined quantities relative to β-actin indicated that expression of all the parameters examined was low on d 15 and 17, increased 9- to 25-fold on d 19, and all decreased again on the day of hatch. The relative expression of mRNA of the different enzymes and transporters were correlated (r = 0.75 to 0.96), but expression was not correlated with the enzymatic activities.

DISCUSSION

At hatch the yolk represents the major nutrient supply to the hatchling until transition to utilization of exogenous feed occurs. Prior to hatch part of the yolk is transported to the small intestine via the yolk stalk and is conveyed to the upper small intestine by antiperistaltic contractions of the small intestine where digestion initially by pancreatic and finally by brush border hydrolases takes place (Geyra et al., 2001). The ontogeny of these processes in late embryonic development has not been described, although posthatch changes have been documented (Black, 1978; Noy and Sklan, 1995; Uni et al., 1998, 1999).

The embryonic pancreas develops the capacity to secrete proteolytic enzymes before hatching; the specific activities of carboxypeptidase A and chymotrypsin progressively increase from 16 d of incubation until a maximum 2 d after hatching (Marchaim and Kulka, 1967). Pancreatic α-amylase was detected by Marchaim and Kulka (1967) at 18 d of incubation, but maximum specific activity was attained 4 d posthatch. Pancreatic lipase increases linearly until 16 d after hatching and then reaches a plateau. However, lipase is active in yolk digestion prior to hatching (Noy and Sklan, 1998).

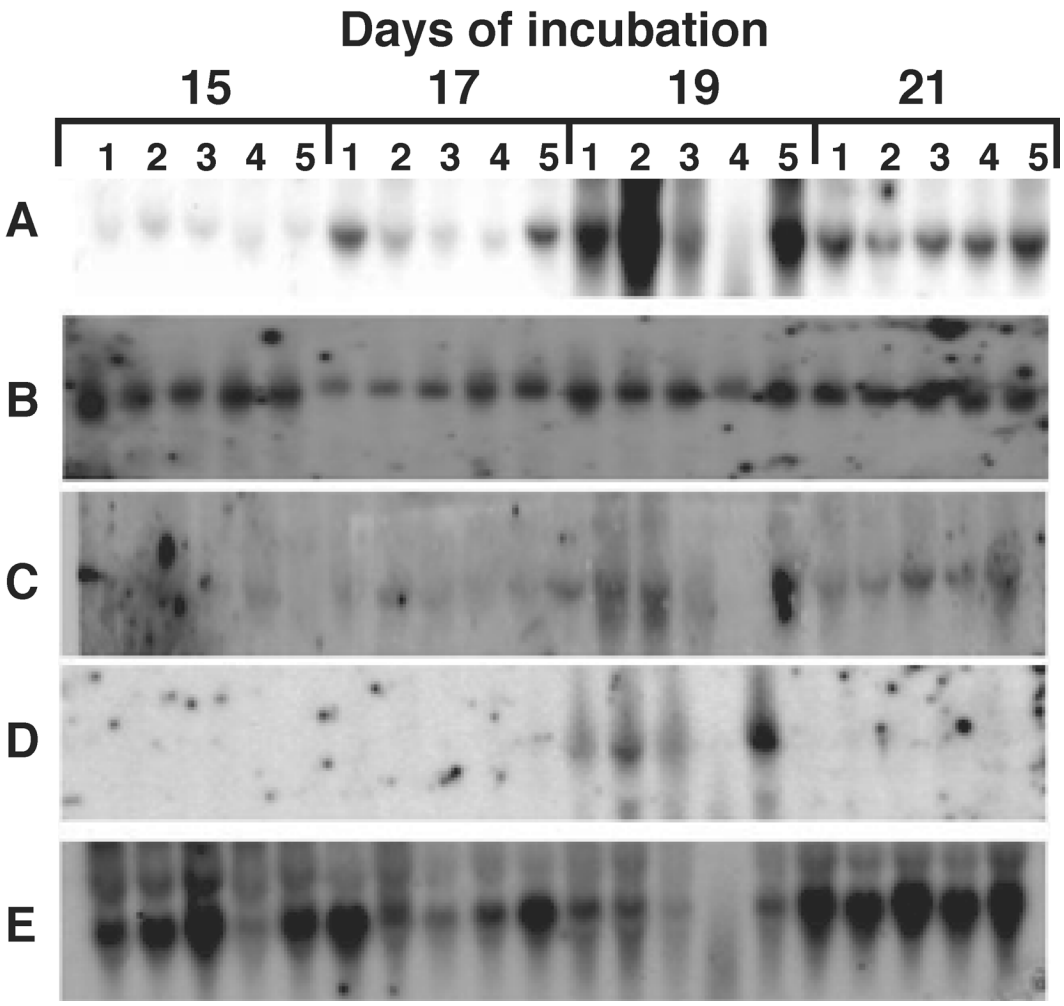

FIGURE 3. Northern blot analysis for mRNA of sucrase-isomaltase (panel A), aminopeptidase (panel B), sodium-glucose transporter-1 (SGLT-1) (panel C), and Na^+K^+ATPase (panel D) from embryonic jejunum (n = 5) at 15, 17, 19 d of incubation and at day of hatch. The same blots were rehybridized with radioactive β-actin as a reference probe (panel E).

Thus, pancreatic enzymes are present and active during embryonic development; brush-border enzymes have also been detected in the embryonic intestine. The ontogeny of SI in mammals has been documented. In the mouse intestine, SI is expressed at low levels late in fetal development when the stratified endoderm cells transform into columnar epithelium with nascent villi (Tung et al., 1997). This low level of SI is maintained until 16 to 17 d postnatal when rapid increases in expression occur preweaning (Boudreau et al., 2001). In chickens, development is clearly different because there is no suckling phase, and complex carbohydrates must be digested and taken up soon after hatch. In chicken embryos, the temporal pattern of SI expression shows activity before hatch and before any carbohydrate is ingested, with a major increase in expression at 19 d incubation. In mammals SI activity is induced by cdx2 and other transcription factors binding to the SI promotor region (Krasinski et al., 2001). It has been shown in the chicken that cdxA, which is similar to mammalian cdx2, binds to the SI promotor region (Sklan et al., 2003). Initiation of this transcription is regulated, at least in part, by cdxA, which is expressed in increasing quantities during this period (Sklan et al., 2003). Expression of cdxA in chicks was found from early embryonic stages when it participates in axial determination during gastulation (Frumkin et al., 1994; Geyra et al., 2002), and both cdxA mRNA and protein concentrations increased in the jejunum with developmental stage, reaching a plateau after hatch. In pre- and postnatal mammals and chicks, concentrations of mRNA of SI and cdx2 were not correlated (Boudreau et al., 2001; Sklan et al., 2003).

In the small intestine of mammals and humans, active transport of glucose has been observed in the fetus, brush-border glucose transport gradually increases with gestational age with a spurt in activity during the final stages of gestation (Ferraris, 2001). SGLT-1 expression has not been found to be influenced by cdx genes in mammals. The major apical transport of glucose in mammals is by SGLT-1, however, GLUT5 is capable of some glucose transport (Ferraris, 2001), and in some birds passive transport has been reported to be major route of transport (Chediack et al., 2001). Expression of SGLT-1 mRNA in developing chick embryos was low until 19 d incubation when a dramatic transient surge in expression was ob-

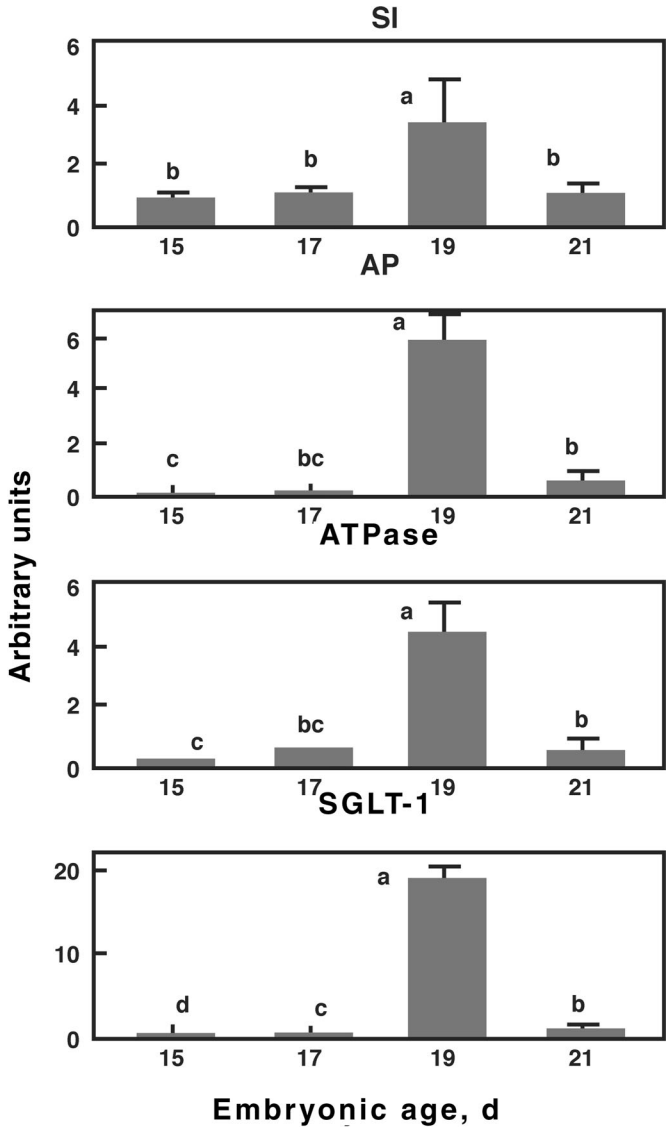

FIGURE 4. The ratio of mRNA expression of sucrase-isomaltase (SI), aminopeptidase (AP), Na^+K^+ATPase, and sodium-gluocse transporter-1 (SGLT-1) relative to β-actin in the embryonic jejunum at 15, 17, 19 d of incubation and at day of hatch. $^{a-c}$Columns with different letters differ significantly ($P < 0.05$). Results are means, and bars are SE from 10 embryos.

served, which decreased by hatch. The pattern of expression of SGLT-1 with development is, however, somewhat different from that of SI. Thus the chick, which must assimilate exogenous carbohydrates soon after hatch, expresses both SI and SGLT mRNA during the late embryonic stages, but the ontogeny of these process appear to be controlled by different mechanisms.

Few studies have examined the ontogeny of ATPase in the chick; however, in the rat ATPase expression was not detected in the small intestine at 16 to 17 d gestation but was observed at cell junctions and in the basal membrane from 18 to 19 d gestation (Amerongen et al., 1989). Activation of the α_1 subunit of the Na^+, K^+-ATPase gene appears to be under the control of multiple protein factors that bind to the negative regulatory regions and appear to regulate transcription activity (Yu et al., 1996). It has been shown that intestinal uptake can be estimated from ATPase activity (Park et al., 1988); however, because activity increases with age (Sklan and Noy, 2000) this comparison should probably be limited to animals of similar developmental stages. Expression of mRNA of jejunal ATPase was detected from 15 d of incubation in the chick and showed a major increase on d 19 before decreasing at hatch; in contrast, ATPase activity increased gradually from d 15.

Limited information is available as to neonatal AP activity. Intestinal transport of amino acids has been reported in chick embryos before hatch and increasing after hatch (Lerner, 1984), and neutral endopeptidase activity has been reported in mouse enterocytes on d 17 (Landry et al., 1994). In this study, expression of mRNA for AP was detected on d 17 of incubation and showed high expression on d 19, which decreased by hatch. Activity increased exponentially from d 15.

Thus, expression of mRNA of all the BBM enzymes and transporters examined followed a similar pattern; in addition, biochemical activities were similar to each other, but the pattern differed from that of the mRNA expression. Although mRNA expression is not necessarily correlated with protein activity, for SGLT1 activity this was reported (Ferraris and Diamond, 1997); however, in this study increases in activity of all enzymes and transporters followed a large increase in expression observed on d 19. Changes in expression of mRNA would be expected to precede changes in activity, which was observed, and, although mRNA expression returned to relatively low levels at hatch, sufficient protein was apparently present to maintain activity levels. It is tempting to speculate that expression is sinusoidal and responds to signals connected to decreases in activity; however, more time points would be needed to determine this. It is clear that the signals triggering activity connected with carbohydrate digestion are occurring much before the system is exposed to dietary carbohydrates.

The major, but transient, acceleration in mRNA expression for all the parameters examined observed on d 19 coincided with a new wave of villus development. Major increases in mRNA expression were not noted in the earlier waves of villus development observed on d 17. Numerically this developmental stage involved an additional growth of 50% in villus size, but this was less than the several-fold increase in RNA expression. Thus additional factors must be involved in the increased expression on d 19. In mammals systemic and luminal factors are involved in gastrointestinal tract development in the prenatal period. Glucocorticoids influence enterocyte differentiation, and in addition, other growth factors and hormones are obtained from amniotic fluid (Connell et al., 1995; Trahair and Sanglid, 1997). In chicken embryos, amniotic fluid is also ingested by the embryo (Romanoff, 1960) and administration of insulin-like growth factor-1 and insulin have specific stimulatory effects on embryonic growth (Christensen et al., 1999; Schmidek et al., 2001). Therefore it can be hypothesized that factors in the am-

nion or in the yolk influence developmental function of the intestine.

Villi lining the avian intestine originate from longitudinal ridges running the length of the embryonic intestine. At 9 d three ridges form due to constrictions mediated by bands of microfilaments with three more appearing about 1 d later (Burgess, 1975). Thus initial formation of villi occurs in several stages, which apparently also characterize the later development observed in this study. Information on chickens is sparse as to the cellular and molecular changes occurring. In the mammalian intestine, morphogenesis includes definition of the crypt-villus axis as well as the duodenal-colon axis, which can be followed by use of molecular markers (Gutierrez et al., 1995). In chickens, the assembly of the villus cytoskeleton during embryogenesis has been documented by following expression of the cytoskeletal proteins, fimbrin, villin, and the 100-kD subunit of the calmodulin protein complex (Shibayama et al., 1987). Substantial increases in concentrations of all three cytoskeletal proteins were observed close to hatch, although cellular location was diffuse. This changed rapidly with localization to the adult pattern occurring between 19 d and hatch. These changes in cytoskeletal proteins are parallel to the major changes in brush-border enzyme and transporter activities close to hatch reported here. It is possible that localization of the proteins to the brush border may temporarily perturb translational control.

Major changes in expression, localization, and ability of functional brush-border proteins thus prepare the framework for posthatch ingestion of carbohydrate-and-protein-rich exogenous feed.

REFERENCES

Amerongen, H. M., J. A. Mack, J. M. Wilson, and M. R. Neutra. 1989. Membrane domains of intestinal epithelial cells: Distribution of Na+,K+-ATPase and the membrane skeleton in adult rat intestine during fetal development and after epithelial isolation. J. Cell. Biol. 109:2129–2138.

Benajiba, A., and S. Maroux. 1980. Purification and characterization of an aminopeptidase A from hog intestinal brush-border membrane. Eur. J. Biochem. 107:381–388.

Black, B. L. 1978. Morphological development of the epithelium of the embryonic chick intestine in culture: influence of thyroxine and hydrocortisone. Am. J. Anat. 153:573–599.

Boudreau, F., Y. Zhu, and P. G. Traber. 2001. Sucrase-isomaltase gene transcription requires the hepatocyte nuclear factor-1 (HNF-1) regulatory element and is regulated by the ratio of HNF-1 alpha to HNF-1 beta. J. Biol. Chem. 276:32122–32128.

Burgess, D. R. 1975. Morphogenesis of intestinal villi. II. Mechanism of formation of previllous ridges. J Embryol. Exp. Morphol. 34:723–740.

Chediack, J. G., E. Caviedes-Vidal, W. H. Karasov, and M. Pestchanker. 2001. Passive absorption of hydrophilic carbohydrate probes by the house sparrow Passer domesticus. J. Exp. Biol. 204:723–731.

Christensen, V. L., J. P. McMurtry, W. E. Donaldson, and K. E. Nestor. 1999. Supplemental oxygen affects plasma insulin-like growth factors in embryos from selected lines of turkeys. Poult. Sci. 78:1606–1610.

Connell, B. J., L. A. Bate, W. Ireland, and R. Acorn. 1995. Morphological changes in the small intestine of the fetal pig after prenatal stimulation of the sow with ACTH. Histol. Histopathol. 10:255–264.

Dahlquist, A. 1964. Method of assay of intestinal disaccaridases. Anal. Biochem. 7:18–25.

Ferraris, R. P., and J. Diamond. 1997. Regulation of intestinal sugar transport. Physiol. Rev. 77:257–302.

Ferraris, R. P. 2001. Dietary and developmental regulation of intestinal sugar transport. Biochem. J. 360:265–276.

Frumkin, A., G. Pillemer, R. Haffner, N. Tarcic, Y. Gruenbaum, and A. Fainsod. 1994. A role for CdxA in gut closure and intestinal epithelia differentiation. Development 120:253–263.

Gal-Garber, O., S. J. Mabjeesh, D. Sklan, and Z. Uni. 2000. Partial sequence and expression of the gene for and activity of the sodium glucose transporter in the small intestine of fed, starved and refed chickens. J. Nutr. 130:2174–2179.

Gal-Garber, O., S. Mabjeesh, D. Sklan, and Z. Uni. 2003. Nutrient transport in the small intestine: Na+,K+-ATPase expression and activity in the chicken small intestine as influenced by dietary sodium. Poult. Sci. 82:1127–1133.

Gal-Garber, O., and Z. Uni. 2000. Chicken intestinal aminopeptidase: partial sequence of the gene, expression and activity. Poult. Sci. 79:41–45.

Geyra, A., Z. Uni, O. Gal-Gerber, D. Guy, and D. Sklan. 2002. Starving affects CDX gene expression during Small Intestinal Development in the Chick. J. Nutr. 132:911–917.

Geyra, A., Z. Uni, and D. Sklan. 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci. 80:776–782.

Gutierrez, E. D., K. J. Grapperhaus, and D. C. Rubin. 1995. Ontogenic regulation of spatial differentiation in the crypt-villus axis of normal and isografted small intestine. Am. J. Physiol. 269:G500–511.

Katanbaf, M. N., E. A. Dunnington, and P. B. Siegel. 1988. Allomorphic relationships from hatching to 56 days in parental lines and F1 crosses of chickens selected over 27 generations for high or low body weight. Growth Dev. Aging 52:11–22.

Krasinski, S. D., H. M. Van Wering, M. R. Tannemaat, and R. J. Grand. 2001. Differential activation of intestinal gene promoters: functional interactions between GATA-5 and HNF-1 alpha. Am. J. Physiol. 281:G69–84.

Landry, C., C. Huet, P. Mangeat, A. Sahuquet, D. Louvard, and P. Crine. 1994. Comparative analysis of neutral endopeptidase (NEP) and villin gene expression during mouse embryogenesis and enterocyte maturation. Differentiation 56:55–65.

Lerner, J. 1984. Cell membrane amino acid transport processes in the domestic fowl (Galluws domesticus). Comp. Biochem. Physiol. A 78:205–215.

Lescale-Matys, L., D. S. Putnam, and A. A. McDonough. 1993. Surplus Na+ pumps: how low-K(+)-incubated LLC-PK1 cells respond to K+ restoration. Am. J. Physiol. 265:C887–892.

Marchaim, U., and R. G. Kulka. 1967. The non-parallel increase of amylase, chymotrypsinogen and procarboxypeptidase in the developing chick pancreas. Biochim. Biophys. Acta 146:553–559.

Noy, Y., and D. Sklan. 1995. Digestion and absorption in the young chick. Poult. Sci. 74:366–373.

Noy, Y., and D. Sklan. 1998. Yolk utilisation in the newly hatched poult. Br. Poult. Sci. 39:446–451.

Palo, P. E., J. L. Sell, F. J. Piquer, L. Vilaseca, and M. F. Soto-Salanova. 1995. Effect of early nutrient restriction on broiler chickens. 2. Performance and digestive enzyme activities. Poult. Sci. 74:1470–1483.

Park, H., B. McBride, L. Milligan, and L. Trouten-Radford. 1988. Acute effects of epidermal growth factor on Na+,K+ ATPase-dependent oxygen consumption and the amount of the enzyme units in enterocytes isolated from the jejunum of chickens. Can. J. Anim. Sci. 78:327–333.

Romanoff, A. 1960. The Avian Embryo. Macmillan, New York.

SAS Institute. 1986. SAS User's Guide. Version 6 ed. SAS Institute Inc., Cary, NC.

Schmidek, A., T. Hare, L. Milakofsky, B. Nibbio, and A. Epple. 2001. Insulin-like growth factor-I affects amino compounds in the fluids of the chicken embryo. Gen. Comp. Endocrinol. 123:235–243.

Sell, J. L., C. R. Angel, F. J. Piquer, E. G. Mallarino, and H. A. al-Batshan. 1991. Developmental patterns of selected characteristics of the gastrointestinal tract of young turkeys. Poult. Sci. 70:1200–1205.

Shibayama, T., J. M. Carboni, and M. S. Mooseker. 1987. Assembly of the intestinal brush border: Appearance and redistribution of microvillar core proteins in developing chick enterocytes. J. Cell Biol. 105:335–344.

Sklan, D. 2001. Development of the digestive tract of poultry. Worlds Poult. Sci. J. 57:415–428.

Sklan, D., A. Getra, E. Tako, O. Gal-Gerber, and Z. Uni. 2003. Ontogeny of brush border carbohydrate digestion and uptake in the chick. Br. J. Nutr. 89:747–753.

Sklan, D., and Y. Noy. 2000. Hydrolysis and absorption in the small intestines of posthatch chicks. Poult. Sci. 79:1306–1310.

Trahair, J., and P. Sanglid. 1997. Systemic and luminal inflences on the perinatal development of the gut. Equine Vet. J. 24(S):40–50.

Tung, J., A. J. Markowitz, D. G. Silberg, and P. G. Traber. 1997. Developmental expression of SI is regulated in transgenic mice by an evolutionarily conserved promoter. Am. J. Physiol. 273:G83–92.

Uni, Z., A. Geyra, H. Ben-Hur, and D. Sklan. 2000. Small intestinal development in the young chick: crypt formation and enterocyte proliferation and migration. Br. Poult. Sci. 41:544–551.

Uni, Z. 1998. Research notes: Identification and isolation of chicken sucrase-isomaltase cDNA sequence. Poult. Sci. 77:140–144.

Uni, Z., S. Ganot, and D. Sklan. 1998. Posthatch development of mucosal function in the broiler small intestine. Poult. Sci. 77:75–82.

Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. Poult. Sci. 74:1622–1629.

Uni, Z., Y. Noy, and D. Sklan. 1999. Posthatch development of small intestinal function in the poult. Poult. Sci. 78:215–222.

Yu, H. Y., S. Nettikadan, D. M. Fambrough, and K. Takeyasu. 1996. Negative transcriptional regulation of the chicken Na+/K(+)-ATPase alpha 1-subunit gene. Biochim. Biophys. Acta 1309:239–252.