Suckling Induces Rapid Intestinal Growth and Changes in Brush Border Digestive Functions of Newborn Pigs[1,2]

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ABSTRACT The interplay between suckling, intestinal growth and brush-border membrane functions is critical during the perinatal period. The present study investigates changes in intestinal dimensions, activities of four brush border membrane hydrolases (lactase, sucrase, maltase and aminooligopeptidase) and rates of sugar and amino acid uptake by intact tissues and brush border membrane vesicles during the first 24 h of suckling. Total intestinal weight, mucosal weight and protein content increased 58%, 80% and 126% ($P < 0.05$) during the first 6 h of suckling; length and surface area did not increase. Total mucosal DNA content was 4.6-fold higher at 24 h after birth, with the rate of increase differing among intestinal regions. Hydrolytic capacities of the entire small intestine increased, more so for homogenates than for brush border membrane vesicles, and more for lactase relative to the other hydrolases studied. Rates of nutrient transport declined, especially for brush border membrane vesicles, for proximal and mid-intestine relative to distal intestine, and for glucose relative to galactose and amino acids. We conclude that *1*) changes in brush border membrane digestive functions coincide with rapid intestinal growth, with postnatal patterns varying among hydrolases, transporters and regions; *2*) insertion into the brush border membrane, not synthesis, limits the postnatal increase of hydrolase activity; and *3*) despite declines in specific activity, hydrolytic and glucose transport capacities of the entire intestine remained stable or increased, and exceeded estimated dietary loads because of intestinal growth. J. Nutr. 127: 418–426, 1997.

KEY WORDS: • *colostrum* • *neonatal* • *pigs* • *nutrient transport* • *brush border hydrolases*

The interaction of diet, intestinal growth and digestive functions is critical during the perinatal period when mammals switch from placental to enteral nutrition. The onset of suckling triggers rapid postnatal intestinal growth in several species [e.g., rats (Berseth et al. 1983), rabbits (Gall and Chung 1982) and dogs (Schwarz and Heird 1994)], but the lack of intestinal growth in kittens during the first week after birth (Buddington and Diamond 1992) indicates the response is not universal. In pigs, the rapid postnatal intestinal growth elicited by colostrum (Widdowson et al. 1976) has been attributed to endocytosis of ingested immunoglobulins, mucosal hyperplasia and protein synthesis (Burrin et al. 1992, Simmen et al. 1990). It is accompanied by changes in intestinal morphology (Xu et al. 1992) and enterocyte ultrastructure (Komuves et al. 1993).

Much less is known about the consequences of rapid postnatal intestinal growth on intestinal functions. The best known example is for rats at weaning, when the rapid proliferation of enterocytes coincides with the appearance of sucrase activity and fructose transport (Henning 1987, Toloza and Diamond 1992). Even though lactase specific activity declines during this period, total intestinal lactase activity remains relatively stable because of intestinal growth (Montgomery et al. 1991). Several changes also accompany the rapid perinatal intestinal growth in pigs. These include declines in rates of monosaccharide transport (Puchal and Buddington 1992), shifts in the processing of lactase gene products (Burrin et al. 1994), changes in brush border membrane (BBM)[4] physical and chemical properties (Alessandri et al. 1990), and the loss of endocytic functions (Westrom et al. 1989).

The present study defines the effect of feeding during the first 24 h after birth when the pig intestine undergoes dramatic changes in structure and functions. This was accomplished by correlating intestinal growth with functional properties. A related objective was to understand the effect of intestinal growth on the functional capacities of the entire length of small intestine. This was accomplished by measuring intestinal dimensions, protein and DNA content, rates of nutrient transport, and the activities of four BBM hydrolases [lactase, sucrase, maltase and aminooligopeptidase (AOP)]. Because measurements were made at three sites along the entire length of small intestine at four ages between birth and 24 h, our results provide better resolution of spatial and temporal changes and

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[4] Abbreviations used: AOP, aminooligopeptidase; BBM, brush border membrane; BBMV, brush border membrane vesicles; NS, not significant.

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the associated correlations than in previous studies that evaluated either fewer or more distant time points and in most cases only one region of the intestine. We provide much needed data about changes in nutrient uptake during the neonatal period, and whereas there are numerous studies of the disaccharidases, relatively little is known about AOP and other peptidases, despite their importance in protein digestion. Pigs were chosen as the model because of the relevance for studying digestion in humans (Moughan et al. 1992).

MATERIALS AND METHODS

Chemicals. All salts and chemicals used to prepare solutions were of the highest purity available. Radioisotopes were purchased from Du Pont NEN Research Products (Mississauga, Ontario, Canada) and American Radiolabelled Chemicals (St. Louis, MO).

Experimental animals and collection of tissues. We purchased from a nearby commercial producer (A. B. Forte, West Point, MS) 15 crossbred standard farm pigs of both sexes that originated from a total of five litters, none of which had more than eight pigs. All experimental procedures using animals were approved by the Mississippi State University Institutional Animal Care and Use Committee. Comparable-sized newborn pigs in each litter were randomly assigned to one of four age groups (0, 6, 12 and 24 h of suckling), with no litter contributing more than one animal to a single age group. Four of the pigs were removed from the sow immediately after birth and before suckling and were studied within 1 h. Another two groups of four pigs were allowed to suckle for 6 and 12 h, and the remaining three pigs were collected after 24 h of suckling. The pigs were transported to the lab and killed 45 min after they were removed from the sow (Beuthanasia, Schering-Plough Animal Health, Kenilworth, NJ, 1 mL/kg, given intravenously). Body weights were recorded, and the entire small intestine was removed and placed in cold (2–4°C), aerated (95% O_2:5% CO_2) mammalian Ringer's solution. The associated mesenteries were cut so the intestines could be straightened along a table top and length measured in a relaxed state. The intestines were divided into three regions of equal length (proximal, mid and distal). From the middle of each region a 15- to 20-cm segment was removed for measurements of dimensions and rates of uptake by intact tissues (see below). Mucosa was obtained from the remainder of each region by scraping with a glass slide, and two aliquots were stored at −70°C.

Measurements of intestinal dimensions. From the middle of each region we removed a 10-cm segment. After recording of wet weight, each segment was slit along the length for measurement of circumference, which was used to calculate nominal surface area (without accounting for area amplification by villi and microvilli). These values were used to determine regional weights and surface areas, which were summed to calculate values for the entire intestine. The percentage of mucosa was based on dry weight after gently scraping each segment with a glass slide and drying the mucosa and underlying tissues to a constant weight (48 h, 45–50°C). Mucosal percentages were used to calculate regional and total quantities of mucosa.

Measurements of brush border membrane hydrolase activities. One of the two aliquots of frozen mucosa from each region of each pig was used to prepare brush border membrane vesicles (BBMV) by $CaCl_2$ precipitation (Schmitz et al. 1973), which is reported to yield higher enzyme recovery than $MgCl_2$ (Ibrahim and Balasubramanian 1995). The BBMV were suspended in 20 mmol/L Tris-HEPES–100 mmol/L mannitol buffer (pH 7.5), and protein content of homogenates and BBMV was determined by the Coomassie Blue method (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as the standard.

Lactase (EC 3.2.1.23), sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20) activities associated with the homogenates and BBMV were determined by the method of Dahlqvist (1964); AOP (EC 3.4.11.2) activity was assayed by the method of Wojnarowska and Gray (1975) using 0.17 mmol/L leucyl-β-naphthylamide. In addition, we measured lactase and sucrase activities in the different fractions that resulted during preparation of BBMV using mucosa from the three regions of pigs suckled for 6 h ($n = 4$). Enzyme activities [micromoles of substrate hydrolyzed per minute (IU)] were normalized to protein content. Total intestinal hydrolase activities (millimoles hydrolyzed per minute) were calculated by summing the hydrolytic capacities of each region (product of BBMV/homogenate specific activity in each region × BBMV/homogenate protein content per gram mucosa × regional mucosal weight).

Measurements of DNA content. A microfluorometric method (Cesarone et al. 1979) was used to quantify DNA content. Aliquots of mucosal homogenates were added to 33258 Hoechst fluorochrome (Sigma Chemical, St. Louis, MO), which was dissolved in water and had a final concentration of 1.5 μmol/L. Fluorescent readings were recorded using excitation and emission wavelengths of 360 and 450 nm, respectively, and compared with a standard prepared with calf thymus DNA.

Measurements of initial rates of brush border membrane vesicle hexose and amino acid uptake. Studies of BBMV transport followed the same methods used to define glucose and amino acid transport by fetal and neonatal pigs (Buddington and Malo 1996). Specifically, BBMV were prepared from the second frozen aliquots of mucosa by $MgCl_2$ precipitation and suspended in 50 mmol/L Tris-HEPES buffer (pH 7.5) with 0.1 mmol/L $MgSO_4$, 200 mmol/L KCl and 125 mmol/L mannitol. Aliquots (25 μL, 10–40 g/L) were stored in liquid nitrogen until used for transport measurements (within 48 h of final BBMV preparation). Initial rates of uptake were measured at 25°C using a rapid filtration technique and a fast sampling, rapid filtration apparatus. On the basis of our previous studies (Buddington and Malo 1996), a total of nine time points were used over 4.5 s for glucose and 20 s for the amino acids. Tracer levels of ^{3}H-labeled D-glucose and L-amino acids and liquid scintillation counting were used to quantify accumulation of glucose and amino acids by BBMV. Final concentrations in the incubation media were 50 mmol/L Tris-HEPES buffer (pH 7.5) with 0.1 mmol/L $MgSO_4$, 96 mmol/L NaCl, 104 mmol/L KCl, 125 mmol/L D-mannitol with 4 μmol/L [^{3}H]D-aldohexose or 2 μmol/L [^{3}H]L-amino acid.

Characterizing kinetics of Na^+/D-glucose cotransport using intact tissues. Rates of uptake by 0.51-cm^2 intact tissues from each region were measured as described previously (Puchal and Buddington 1992). Briefly, pieces of tissues were secured by silk ligatures onto the tips of 0.5-cm stainless steel rods with the mucosa exposed. Beginning 45 min after death, the tissues were preincubated for 5 min in aerated, 37°C mammalian Ringer's solution. They were then exposed for 2 min to 37°C Ringer's solution with 0.1, 1, 5, 10, 25 and 50 mmol/L D-glucose with tracer levels of ^{14}C-labeled D-glucose and ^{3}H-labeled L-glucose. Each solution was aerated and stirred by a bar rotating at 1200 rpm to reduce unstirred layer influences and maintain tissue oxygenation. The tissues were rinsed for 20 s in cold Ringer's solution, removed from the rods and placed in tared vials; wet weight was recorded. Accumulation of radioactive D- and L-glucose by the tissues was measured using liquid scintillation counting. Rates of uptake were calculated and normalized to tissue wet weight; rates of uptake based on tissue surface area yielded similar conclusions. Maximum total intestinal glucose transport capacities (millimoles per minute) were calculated as the sum of the regional transport capacities (product of rates of transport at 50 mmol/L in each region times regional wet weight).

Statistics. Data presented in tables and figures are means ± SEM. We used two-way ANOVA (SAS 1992) to detect effects of age and region on measured variables, with $P < 0.05$ accepted as the critical value. When a significant effect was detected, Duncan's multiple range test (Zar 1974) was used to identify specific differences between ages and/or regions. In the figures, we indicate significant differences between 6, 12 and 24 h suckled pigs in comparison with 0 h pigs, but in the tables we also indicate where differences were detected among the four age groups. Regional comparisons are described in the text of the results.

Kinetics of D-glucose transport by intact tissues was determined by nonlinear regression analysis (Michaelis-Menten) using the Enzfitter software package (Robin G. Leatherbarrow, copyright 1987, Biosoft Elsevier, Amsterdam, The Netherlands) and model equations for one and two transport systems. Initial rates of hexose and amino acid accumulation by BBMV were also determined using the Enzfitter software package. Linear regression analysis was used over the linear part of the uptake-time curves. When uptake-time curves deviated from linearity, a second-degree polynomial analysis was used with the

TABLE 1

Quantitative features of the small intestines of pigs suckled by sows for 0, 6, 12 and 24 h after birth[1]

Variable	Hours of suckling 0 (n = 4)	6 (n = 4)	12 (n = 4)	24 (n = 3)	P value[2]
Body weight, *g*	1466.5 ± 14.5	1546.2 ± 114.0	1568.5 ± 53.6	1713.3 ± 189.8	0.40
Intestinal length, *cm*	319.0 ± 19.4	353.5 ± 12.5	358.2 ± 7.4	352.3 ± 18.0	0.34
Intestinal weight, *g*	47.4 ± 4.0[a]	74.9 ± 5.1[b]	78.5 ± 4.9[b]	83.3 ± 12.6[b]	0.009
Proximal	16.0 ± 1.7[a]	22.5 ± 1.1[ab]	25.7 ± 2.7[ab]	28.3 ± 5.6[b]	0.037
Mid	15.2 ± 0.8[a]	25.5 ± 1.9[b]	29.3 ± 1.3[b]	28.3 ± 3.1[b]	0.0002
Distal	16.1 ± 1.6[a]	26.9 ± 2.2[b]	23.5 ± 1.4[ab]	26.8 ± 4.9[b]	0.030
Intestinal weight/body weight, *g/kg*	32.3 ± 2.9[a]	49.0 ± 4.3[b]	50.1 ± 3.0[b]	48.3 ± 2.3[b]	0.008
Mucosal weight, *g*	32.0 ± 3.4[a]	57.6 ± 3.9[b]	61.5 ± 4.0[b]	66.6 ± 11.5[b]	0.005
Proximal	11.3 ± 1.5[a]	18.7 ± 0.9[ab]	20.8 ± 2.1[ab]	22.7 ± 5.1[b]	0.046
Mid	10.4 ± 1.0[a]	20.5 ± 1.8[b]	23.8 ± 1.0[b]	23.0 ± 3.2[b]	0.0003
Distal	10.3 ± 1.1[a]	18.4 ± 1.6[ab]	16.9 ± 1.0[ab]	20.9 ± 4.1[b]	0.011
Mucosal weight/intestinal weight, *g/100 g*	67 ± 2[a]	77 ± 2[b]	78 ± 1[b]	79 ± 2[b]	0.003
Mucosal weight/body weight, *g/kg*	21.9 ± 2.5[a]	37.6 ± 3.2[b]	39.2 ± 2.3[b]	38.5 ± 2.7[b]	0.002
Nominal surface area, cm^2	414.7 ± 39.2	498.9 ± 31.0	473.3 ± 17.1	528.0 ± 66.8	0.23
Intestinal thickness,[3] *mm*	1.1 ± 0.02[a]	1.5 ± 0.04[b]	1.7 ± 0.06[b]	1.6 ± 0.04[b]	0.001

[1] Values are means ± SEM within a row. Values with the same superscripts are not significantly different, and values without superscripts are not different from the corresponding value for 0 h pigs ($P > 0.05$). Comparisons are restricted to ages.

[2] P value is from the two-way ANOVA and is for the effect of age on the measured and calculated variables.

[3] Intestinal thickness was calculated by dividing tissue weight by nominal surface area, assuming a tissue density of 1 g/cm^3.

initial rate represented by the first-degree coefficient of the polynomial (Chenu and Berteloot 1993).

RESULTS

Body weights and intestinal dimensions. During the first 24 h of suckling, body weight, intestinal length and total surface area increased 17%, 10% and 27%, respectively, none significantly relative to measurements made at birth (**Table 1**). Estimated tissue thickness increased 45% ($P < 0.001$ relative to birth). When normalized to body weight, intestinal length and total surface area did not increase during the 24-h period (data not shown).

Total intestinal weight increased 76% ($P < 0.05$) during the first 24 h, with 96% of the gain due to the mucosa. Furthermore, most of the 24 h weight gain occurred during the first 6 h of suckling, with significant increases in the total weights of the mid and distal intestine ($P < 0.05$) and mucosal weight of the mid intestine ($P < 0.05$). At 24 h, total and mucosal weights were significantly higher than those at birth for all three regions ($P < 0.05$). Weights of the 0.51-cm^2 intact tissues used to measure glucose transport also increased significantly in proximal and mid intestine ($P < 0.001$), providing corroborative evidence of age differences in tissue weight (data not shown).

Protein content of homogenates and brush border membrane vesicles. Total mucosal homogenate protein, which represents mucosal protein content of the entire intestine, increased 126% during the first 6 h after birth ($P < 0.05$, **Table 2**), with the greatest magnitude of increases in mid (149%, $P < 0.001$) and distal (135%, $P < 0.005$) intestine; magnitudes of increases did not differ significantly between regions. Increases in total intestinal mucosal protein thereafter were smaller, but still resulted in a significant increase between 6 and 24 h (58%). Mucosal protein concentrations (milligrams per gram of mucosa) increased in all three regions during the first day after birth (data not shown, $P < 0.001$).

Total intestinal BBMV protein content also increased, but the increase was of smaller magnitude (71% increase at 24 h, $P < 0.05$). In contrast to homogenates, BBMV protein concentrations (milligrams per gram of mucosa) declined slightly during the first 12 h of suckling (for proximal and mid, $P < 0.06$), but by 24 h BBMV protein concentrations had recovered in each region to levels comparable to those at birth (data not shown).

Mucosal DNA content. At birth, DNA contents of the proximal and mid intestine were comparable, with slightly lower values for the distal intestine ($P > 0.10$; Table 2). After 12 h of suckling, total DNA content of the entire intestinal mucosa was 177% higher ($P < 0.05$). This increase was caused by the mid and distal regions (204% and 230%, respectively; $P < 0.05$). The total DNA content of mucosa from the proximal intestine also increased but was not significantly greater than the value at birth until 24 h ($P < 0.05$). Total mucosal DNA content at 24 h was over 4.5-fold higher than the values at birth, with comparable increases for all three regions.

Hydrolase activities. Total intestinal activities were higher at 24 h for all four hydrolases (**Figs. 1–4***A* panels), with the increases greater for homogenates relative to BBMV. At birth and 24 h, total intestinal homogenate activities for sucrase, maltase, and AOP averaged 40–50% of values for lactase. Values for the same ratios were lower at 12 h relative to those at birth (significant for maltase and AOP). In contrast, total intestinal BBMV activities for sucrase and maltase were 10–12% of values for lactase and did not vary during the first 24 h. Total BBMV activity for AOP relative to lactase was similar to that of homogenates (40%) and was stable.

Recoveries calculated from the percentage of total homogenate activity present in the BBMV (pooled values from pigs of all four ages) were higher for lactase and AOP (24% and 27%) relative to sucrase and maltase (6% and 10%).

Homogenate specific activities for lactase did not vary with age in any region (Fig. 1*B*). In contrast, there were abrupt declines within 6 h in maltase homogenate activity in all three regions (Fig. 3*B*). Sucrase and AOP were intermediate in that there were significant effects of age for specific activities of homogenates from proximal and mid intestine for AOP (Fig. 4*B*) and only in mid intestine for sucrase (Fig. 2*B*).

TABLE 2

Small intestinal mucosal protein and DNA content of pigs suckled by sows for 0, 6, 12 and 24 h after birth[1]

Variable	Hours of suckling 0 (n = 4)	6 (n = 4)	12 (n = 4)	24 (n = 3)	P value[2]
Mucosal protein, *g*	2.38 ± 0.35[a]	5.38 ± 0.39[b]	6.74 ± 0.51[bc]	8.51 ± 1.71[c]	0.0011
Proximal	0.87 ± 0.18[a]	1.74 ± 0.12[ab]	2.39 ± 0.30[b]	2.81 ± 0.72[b]	0.0176
Mid	0.82 ± 0.14[a]	2.04 ± 0.19[b]	2.63 ± 0.11[bc]	3.22 ± 0.64[c]	0.0004
Distal	0.68 ± 0.12[a]	1.60 ± 0.23[b]	1.74 ± 0.13[b]	2.48 ± 0.44[c]	0.0011
BBMV protein, *g*	0.28 ± 0.03[a]	0.45 ± 0.03[ab]	0.43 ± 0.03[ab]	0.57 ± 0.07[b]	0.0007
Proximal	0.10 ± 0.01	0.14 ± 0.01	0.15 ± 0.02	0.18 ± 0.04	0.0593
Mid	0.10 ± 0.01[a]	0.15 ± 0.01[b]	0.16 ± 0.01[b]	0.19 ± 0.02[b]	0.0004
Distal	0.08 ± 0.01[a]	0.15 ± 0.01[ab]	0.12 ± 0.01[a]	0.20 ± 0.06[b]	0.0147
Mucosal DNA, *mg*	45.0 ± 6.7[a]	64.0 ± 4.6[ab]	124.7 ± 8.6[b]	250.7 ± 52.9[c]	0.0002
Proximal	16.6 ± 2.7[a]	16.6 ± 2.1[a]	34.9 ± 4.5[a]	107.8 ± 30.3[b]	0.0011
Mid	16.5 ± 2.5[a]	25.9 ± 4.9[a]	50.2 ± 5.4[b]	65.8 ± 6.2[c]	0.0001
Distal	12.0 ± 2.7[a]	21.5 ± 3.1[a]	39.6 ± 3.2[b]	77.0 ± 27.1[c]	0.0092
Mucosal protein/DNA, *mg/mg*	52.8 ± 3.5[a]	83.2 ± 3.6[b]	54.5 ± 4.0[a]	34.4 ± 2.9[c]	0.0007
Mucosal DNA/mucosa, *mg/g*	1.38 ± 0.10[a]	1.13 ± 0.12[a]	2.03 ± 0.11[b]	3.71 ± 0.16[c]	0.0001

[1] Values are mean ± SEM within a row. Values with the same superscripts are not significantly different, and values without superscripts are not different from the corresponding value for 0 h pigs ($P < 0.05$). BBMV = brush border membrane vesicles.
[2] P value is from the two-way ANOVA and is for the effect of age on the measured and calculated variables.

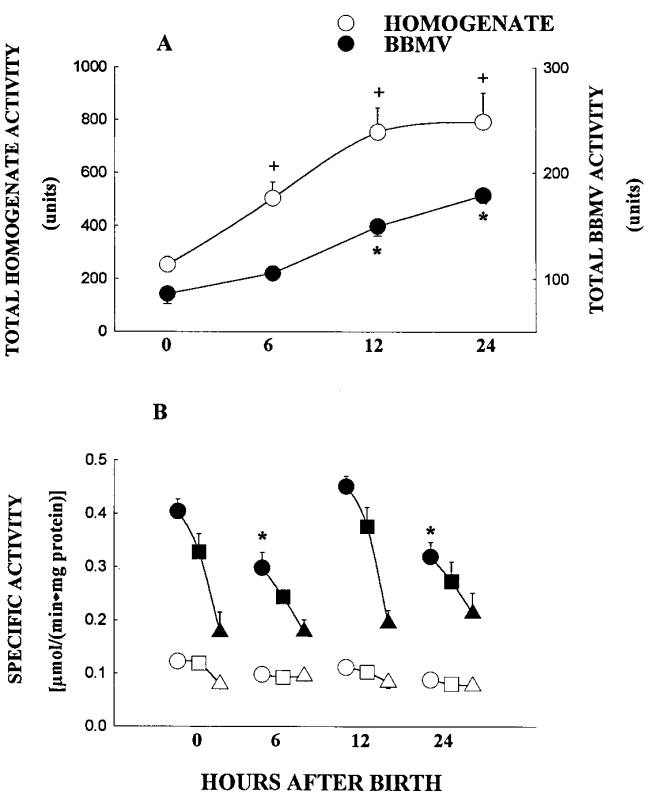

FIGURE 1 Total intestinal lactase activity (units, *panel A*) and specific activity [μmol/(min · mg protein), *panel B*] for homogenates and brush border membrane vesicles (BBMV) prepared from the proximal (circles), mid (squares) and distal (triangles) small intestines of pigs, collected immediately after birth (n = 4) and after suckling for 6 h (n = 4), 12 h (n = 4) and 24 h (n = 3). Values are means ± SEM, with pluses and asterisks indicating significant differences from values for 0 h pigs for homogenates and BBMV, respectively. Total activity was calculated by summing the products of specific activity times protein content in each region.

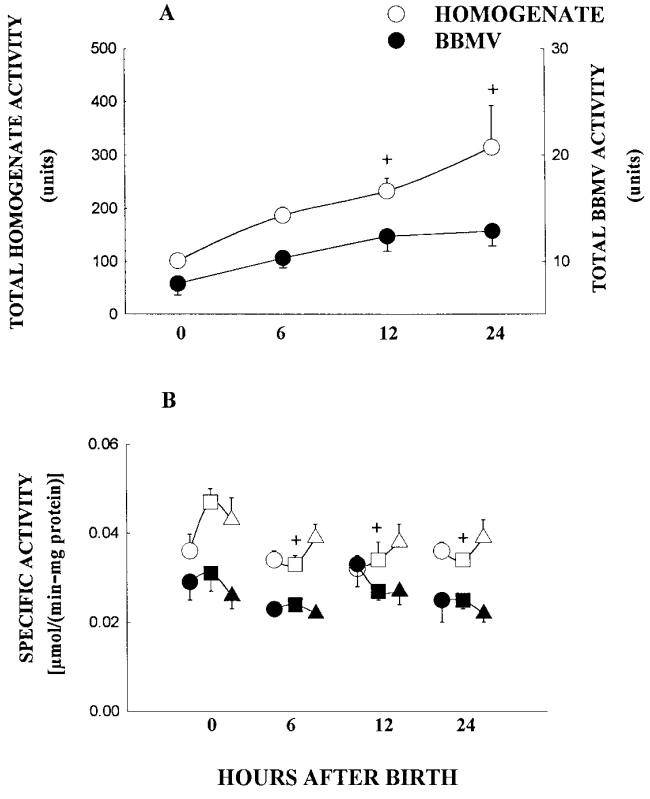

FIGURE 2 Total intestinal sucrase activity (units, *panel A*) and specific activity [μmol/(min · mg protein), *panel B*] for homogenates and brush border membrane vesicles (BBMV) prepared from the proximal (circles), mid (squares) and distal (triangles) small intestines of pigs, collected immediately after birth (n = 4) and after suckling for 6 h (n = 4), 12 h (n = 4) and 24 h (n = 3). Values are means ± SEM, with pluses and asterisks indicating significant differences from values for 0 h pigs for homogenates and BBMV, respectively. Total activity was calculated by summing the products of specific activity times protein content in each region.

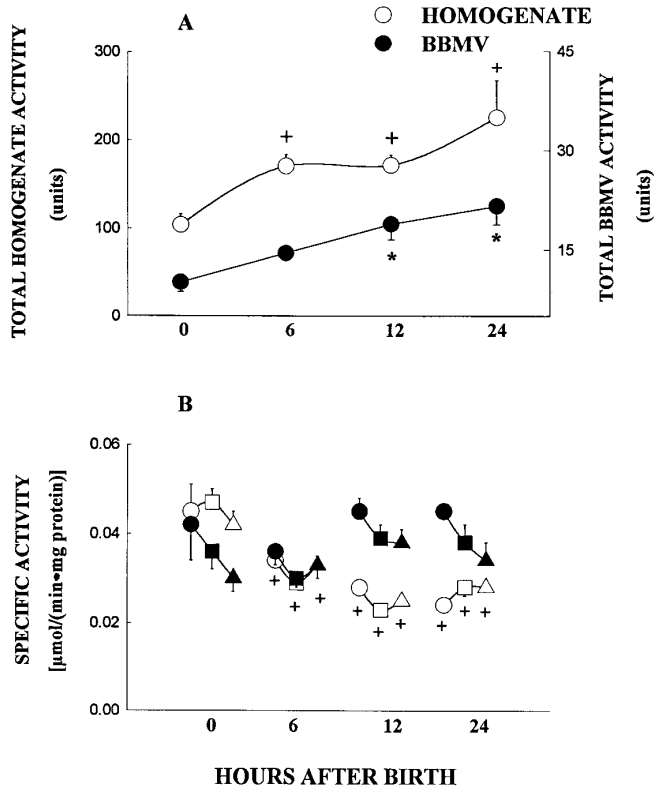

FIGURE 3 Total intestinal maltase activity (units, *panel A*) and specific activity [μmol/(min · mg protein), *panel B*] for homogenates and brush border membrane vesicles (BBMV) prepared from the proximal (circles), mid (squares) and distal (triangles) small intestines of pigs, collected immediately after birth (n = 4) and after suckling for 6 h (n = 4), 12 h (n = 4) and 24 h (n = 3). Values are means ± SEM, with pluses and asterisks indicating significant differences from values for 0 h pigs for homogenates and BBMV, respectively. Total activity was calculated by summing the products of specific activity times protein content in each region.

Brush border membrane vesicle specific activities for all four hydrolases tended to be lower at 6 h but had recovered at 12 h to levels comparable to or exceeding those at birth (Fig. 1–4*B* panels). There was a subsequent decline between 12 and 24 h for all four. When all age groups were considered, the trend was significant for lactase in the proximal ($P < 0.01$) and mid ($P < 0.05$) regions and nearly so for AOP in mid and distal regions ($P < 0.07$ and 0.08, respectively). The trend was not significant for sucrase and maltase in any region (all $P > 0.25$).

During the first 24 h after birth, enrichment factors (calculated as the ratio of specific activities in BBMV relative to homogenates) never exceeded 1 for sucrase; for maltase, values were less than 1 at birth and increased to 1.5 and 1.7 at 12 and 24 h. Values for lactase and AOP were greater than 2, with the highest recorded for AOP in mid intestine at 12 h (5.2).

Measurements of lactase and sucrase activities in the different fractions showed that after the initial low speed centrifugation (2500 × *g*), which sediments unbroken cells and organelles, 81% of sucrase activity in the homogenates was recovered in the supernatants (average of three regions). After the high speed centrifugation (43,200 × *g*), only 19% of the original homogenate activity was recovered in the BBMV pellet, with 51% of the activity associated with the supernatant. In contrast, 34% of homogenate lactase activity was recovered in the BBMV and only 11% remained in the associated supernatant.

Initial rates of aldohexose and amino acid uptake by brush border membrane vesicles. Glucose uptake by BBMV declined dramatically after 6 h of suckling in all three regions (**Fig. 5**). Because of a continuing decline, BBMV uptake by proximal intestine from 24-h-old pigs was only 3% of that at birth. Postnatal declines in uptake, when present, seemed to be of lower magnitude for galactose and the amino acids leucine, proline, aspartate and lysine.

A strong proximal-to-distal gradient for uptake was detected at birth for glucose, but not for galactose or the amino acids. Regional difference in glucose uptake were less pronounced at 6 h and were not evident at 12 and 24 h.

Na^+/D-glucose cotransport by intact tissues. The capacities of the entire length of small intestine to transport glucose (measured at the saturating concentration of 50 mmol/L) did not change appreciably during the first 24 h after birth (**Fig. 6***A*). This reflected a combination of increasing intestinal weight and decreasing rates of glucose uptake per milligram of intestine after onset of suckling.

Kinetic analysis of glucose uptake data using a model for a single transporter showed age and regional differences in V_{max} (Fig. 6*B*); apparent K_m values averaged 1.4 ± 0.1 mmol/L and did not differ among ages or regions. Similar to the BBMV data, glucose uptake by intact tissues declined from proximal to distal (Fig. 6*B*). Rates of uptake in proximal and mid intestine

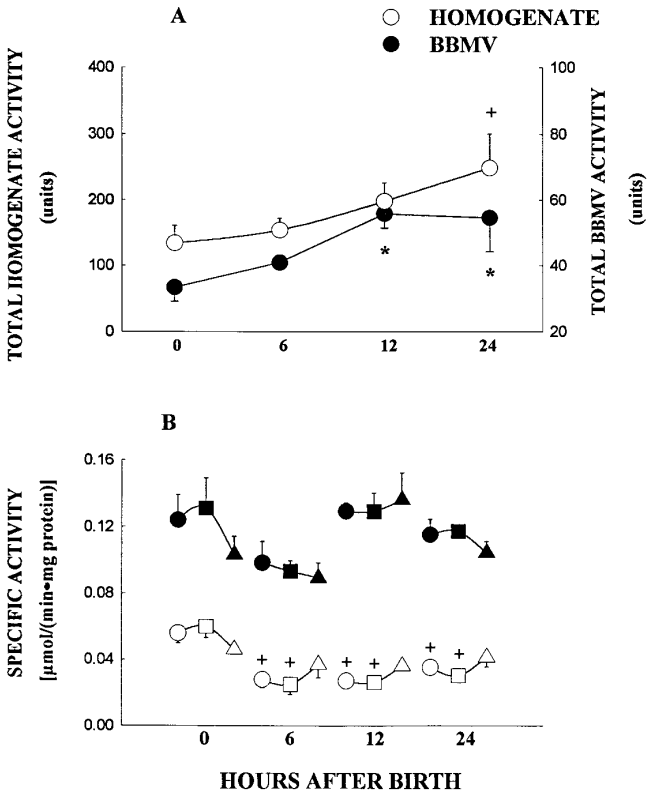

FIGURE 4 Total intestinal aminooligopeptidase activity (units, *panel A*) and specific activity [μmol/(min · mg protein), *panel B*] for homogenates and brush border membrane vesicles (BBMV) prepared from the proximal (circles), mid (squares) and distal (triangles) small intestines of pigs, collected immediately after birth (n = 4) and after suckling for 6 h (n = 4), 12 h (n = 4) and 24 h (n = 3). Values are means ± SEM, with pluses and asterisks indicating significant differences from values for 0 h pigs for homogenates and BBMV, respectively. Total activity was calculated by summing the products of specific activity times protein content in each region.

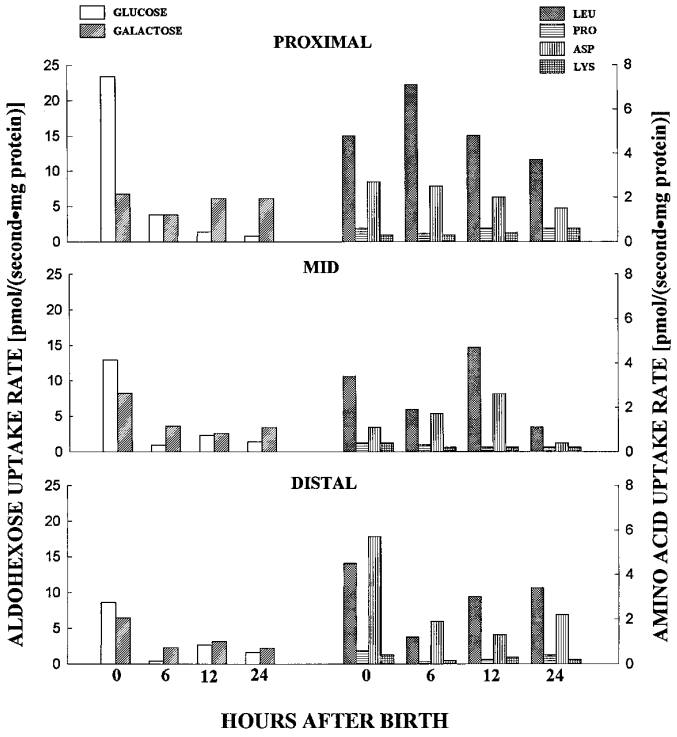

FIGURE 5 Aldohexose and amino acid uptake [pmol/(s · mg protein)] by brush border membrane vesicles prepared from the proximal, mid and distal small intestines of pigs, collected immediately after birth and after suckling for 6, 12 and 24 h (n = 2 for each age).

tended to decline between birth and 24 h, whereas those in distal intestine were stable.

Although it was possible to fit data for the proximal intestine of unsuckled neonates to a model for two transporters, it was not a significant improvement over the model for a single transporter. At no other age or in any other region could data be fit to a two transporter model.

DISCUSSION

Although events before birth prepare the intestine for the transition from placental to enteral nutrition, postnatal growth and maturation of the intestine are essential to match qualitative and quantitative changes in the diet. Our results show that most of the dramatic changes in intestinal structure and functions reported at 24 h after birth occur within the first 6 h of suckling.

Intestinal dimensions and structural characteristics. The percentage of birth weight represented by intestine varies among pigs (present study vs. data of Moughan et al. 1992, Widdowson et al. 1976, Xu et al. 1992). However, the gain in intestinal weight as a percentage of body weight during the first 24 h after birth is comparable. The present results show that most of the growth occurs during the first 6 h of suckling and is caused by a rapid increase in the mucosal component of all three regions. This is evident from the increase in gut thickness (Table 1), which results in a 100% increase in absorptive area at the microscopic level (Burrin et al. 1994, Xu et al. 1992) and greatly exceeds the 27% increase in nominal surface area. Although onset of suckling induces similar rapid weight gains in neonates of other species [rats (Berseth et al. 1983), rabbits (Gall and Chung 1982), dogs (Schwarz and Heird 1994)], this influence differs from the low rates of mucosal weight gain in guinea pigs (Weaver and Lucas 1987) and cats during the first week after birth (Buddington 1992).

Protein content. Mucosal weight gains were accompanied by increases in protein content of the homogenates. This can be attributed to a combination of three factors. First, endocytosis of colostral immunoglobulins, mainly immunoglobulin G (Kiriyama 1992), and nonselective endocytosis of lumenal solutes (Ekstrom and Westrom 1991) would increase homogenate protein content. These activities may be enhanced by protease inhibitors present in colostrum (Lindberg 1982) and the higher protein content of colostrum (13% at birth vs. 6.4% 24 h later; Klobasa et al. 1987). The resulting formation of protein-dense intracellular granules in neonatal enterocytes coincides with changes in cell ultrastructure and a transient epithelial swelling (Xu et al. 1992, Komuves et al. 1993). Although endocytosis and transfer of immunoglobulins to the circulation cease shortly after pigs are born (Westrom et al. 1989), the granules remain for days (Komuves et al. 1993). Second, colostrum increases concentrations of intestinal RNA (Simmen et al. 1990) and protein synthesis by enterocytes that line the intestine at birth (Burrin et al. 1992). Third, higher DNA content at 24 h (Xu et al. 1992, present study) indicates that colostrum stimulates crypt cell proliferation. In preliminary studies using 5-bromo-2′-deoxyuridine, we have confirmed

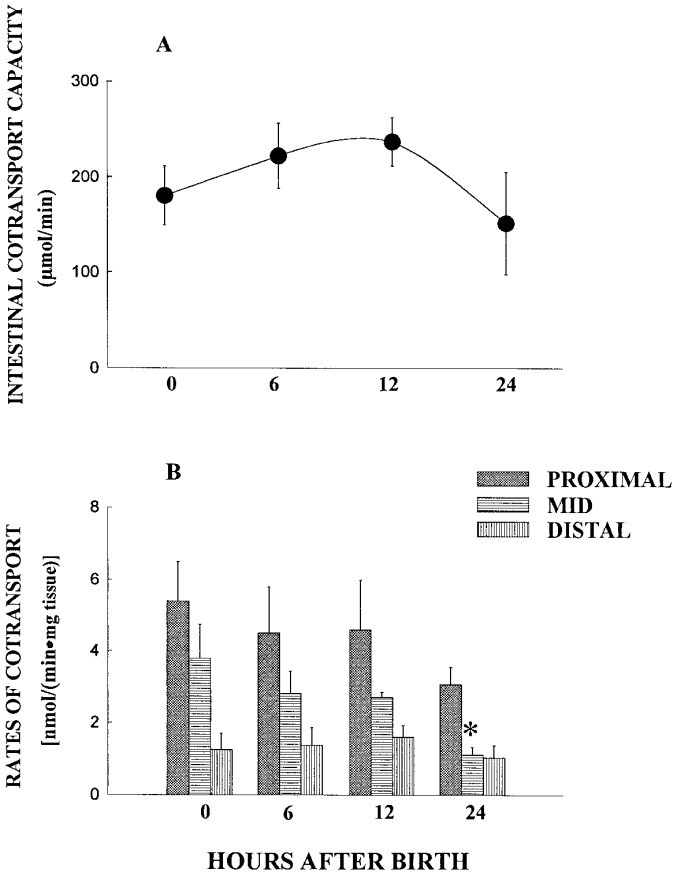

FIGURE 6 Glucose cotransport by intact tissues from the proximal, mid and distal small intestines of pigs, collected immediately after birth (n = 4) and after suckling for 6 h (n = 4), 12 h (n = 4) and 24 h (n = 3). *Panel A:* Intestinal Na^+/D-glucose cotransport capacity (μmol/min). *Panel B:* Maximum rates of cotransport [nmol/(min · mg tissue) at 50 mmol/L glucose]. The asterisk indicates a difference from the value for 0 h pigs. Transport capacities were calculated by summing the products of maximum rates of uptake in each region times total regional wet weight.

there is an increase in cell proliferation after 6 h of suckling compared with unsuckled neonates (unpublished data). The different age-related patterns of DNA increase observed for the three regions show there are both spatial and temporal differences in rates of enterocyte proliferation during the first 24 h after birth. The onset of suckling immediately stimulates enterocyte proliferation in the mid and distal small intestine, but the response is delayed in the proximal intestine until after 6 h. An unknown fraction of the increased mucosal DNA would be contributed by adherent bacteria that rapidly colonize the intestine after birth (Swords et al. 1993).

The higher total BBMV protein content at 24 h may be caused by a combination of an increase in the amount of BBM, insertion of synthesized proteins, and binding of colostral proteins to receptors present in the BBM. It is also possible that because of endocytosis during the perinatal period, an unknown percentage of the isolated BBMV may have been apical membrane that had been internalized.

During the first hours after birth there is a proportionally greater accumulation of protein than DNA in the intestine, resulting at 6 h in a 60% increase in the ratio of protein relative to DNA. Thereafter, DNA content increases faster and the ratio of protein to DNA at 24 h is 35% less than the value at birth. These changes and the nearly exponential increase in mucosal DNA between birth and 24 h clearly show that rapid neonatal intestinal growth is caused by a combination of hyperplasia and hypertrophy.

Functional characteristics. In addition to the postnatal changes in hydrolase activities and rates of nutrient transport (present study, Burrin et al. 1992, Puchal and Buddington 1992, Schwarz and Heird 1994), there are shifts in other intestinal functions of pigs. These include declines in the number of receptors for epidermal growth factor (Kelly et al. 1992), loss of receptor-mediated transcytosis of colostral immunoglobulins (Westrom et al. 1989) and an increase in the concentrations of fatty acid binding proteins (Reinhart et al. 1992). The earlier and more dramatic changes in hydrolase activities and rates of transport by proximal intestine correspond with an earlier loss of endocytic capacities relative to distal intestine (24 h vs 6 d; Ekstrom and Westrom 1991) and reflect the proximal to distal pattern of maturation. These changes are not unique to pigs and are reported for other species with rapid postnatal intestinal growth (Buddington 1994, Schwarz and Heird 1994).

In pigs and other species, colostrum accelerates enterocyte proliferation and maturation of the intestine (Jaeger et al. 1987, Koldovsky et al. 1992), increases transport of electrolytes and nutrients (Bird et al. 1994), stimulates secretion of glucoregulatory hormones (Burrin 1992, Lepine et al. 1989) and influences later development of the intestine (Kelly et al. 1993). It is commonly thought these influences are mediated by the high concentrations of various growth factors. However, nutrients in colostrum may also be influential, whether directly or indirectly by stimulating secretions.

Of critical importance to developing animals is whether postnatal intestinal growth and changes in activities of hydrolases and rates of absorption have any affect on digestive capacities. Despite postnatal declines in lactase specific activity (present study, Burrin et al. 1994) and rates of nutrient transport per milligram of tissue, total intestinal lactase activity remains stable (Montgomery et al. 1991) or increases (present study), and transport capacities for most nutrients increase (Buddington 1992). For example, total lactase activity associated with the BBMV of newborn pigs is sufficient to digest 1.7 g of lactose in 1 h, exceeding the 0.34 g of lactose in the 10 g of colostrum (Widdowson 1985) consumed by newborn pigs (our unpublished observations). Similarly, total lactase activities in the proximal intestine of neonatal puppies (0.25 g/h; Schwarz and Heird 1994) are sufficient to hydrolyze estimated dietary loads (0.15 g; based on consumption of 5 mL milk/h with 3% lactose; Jenness and Sloan 1970). The postnatal increase in total intestinal lactase activity should be more than enough to compensate for increases in milk consumption by pigs during the first 24 h after birth.

The detection of maltase and sucrase at birth (present study, Manners and Stevens 1972), albeit at low activities, suggests pigs are provided with a limited capacity to process alternative sources of carbohydrate. Humans are also born with maltase and sucrase (Auricchio and Sebastio 1989), and this may be an important adaptation that allows neonates of both species to digest foods other than milk and be weaned at or shortly after birth (Greer and Apple 1991, Leibbrandt et al. 1977). Corresponding with the ability of the sucrase-isomaltase and maltase/glucoamylase complexes to hydrolyze maltose, maltase specific activity was higher than that of sucrase. However, diarrhea results when dietary loads exceed total intestinal maltase and sucrase activities, such as we found when feeding neonatal pigs milk replacers high in sucrose. It is difficult to calculate dietary loads of substrates for AOP. However, the presence of AOP in neonatal pigs (present study, Tarvid et al. 1994) and subsequent increase after onset of suckling suggest it is important for hydrolyzing and making available components of milk.

The low enrichments for all four BBM hydrolases, and in some cases even higher activity in homogenates relative to BBMV, are unexplained. We found using the same methods that lactase enrichment values are much higher during gestation (16-fold for average of 7, 8, 10, and 12 wk fetuses; Buddington and Malo 1996) and for 10 d sucklings (13-fold), and adults (eight-fold, but 13-fold for sucrase), with lactase enrichments at 3 d intermediate (4.9-fold, unpublished data). These findings and the low enrichments for unsuckled neonates (present study, Buddington and Malo 1996) indicate there are changes late in gestation and during the first postnatal days in the insertion of hydrolases into the BBM. The apparent presence of soluble lactase and sucrase, as revealed by assaying the different fractions during BBMV preparation, corresponds with previous reports of higher proportions of soluble BBM hydrolases during early postnatal development (Galand and Forstner 1974, Reisenauer et al. 1992, Seetharam et al. 1977). The lower recovery of sucrase might be related to a greater proportion of soluble sucrase in neonates relative to adults. This might be caused by a weaker attachment of sucrase to the sucrase-isomaltase complex or the entire complex to the BBM, and possible intracellular activation of the enzyme.

Much less is known about rates of glucose and amino acid transport during neonatal development. Postnatal intestinal hypertrophy and hyperplasia can cause declines in rates of uptake normalized to tissue mass, even though density and functions of transporters remain unchanged. In addition, villus swelling might restrict the diffusion of nutrients down to enterocytes lower on the villi, thus effectively reducing absorptive surface area and rates of transport (Strocchi and Levitt 1993). Furthermore, there is a redistribution of transport from along the entire crypt-to-villus axis at birth (Smith 1988), to the tips of villi, as reported for the distribution of Na^+/glucose cotransporters in older animals (Freeman et al. 1993, Hwang et al. 1991). The lack of decline in glucose transport by intact distal intestine, despite greater increases in tissue weight and changes in villus architecture, indicates there was either synthesis and insertion of new transporters or increased activity of those existing at birth.

Comparisons of total lactase activity with total glucose transport capacities show that the two functions are matched at birth, with the amounts of glucose transported per hour averaging 92% (± 15%) of the aldohexose that could be liberated from lactose. On the basis of results from BBMV uptake studies, galactose uptake would be sufficient to handle dietary loads.

Mechanisms. The postnatal changes in hydrolase and transporter activities may be caused by shifts in the genes that are being expressed (Perozzi et al. 1993) and the processing of gene products (Burrin et al. 1994). Colostrum induces synthesis and processing of lactase (Burrin et al. 1992) and, in the present study, was associated with an increase in total intestinal mucosal lactase activity. Increases in total intestinal mucosal activities for maltase, sucrase, and AOP provide further evidence that birth and onset of suckling induce synthesis and processing of BBM hydrolases. What is less clear is whether the changes in gene expression reflect reprogramming of existing enterocytes or the production of enterocytes with adult characteristics that eventually replace the fetal enterocytes (Smith 1988).

The reasons for the more marked decline in rates of glucose uptake by BBMV relative to intact tissues are still unknown. They are puzzling because BBMV from 24-h-old pigs were capable of accumulating galactose and amino acids, indicating the BBMV were functional and the changes were specific for glucose. The declines may be related to changes in the physical and chemical characteristics of the BBM that occur after birth in mammals (Alessandri et al. 1990). These could influence uptake by altering the functions of individual transporters in isolated BBMV, reduce recovery of BBM with transporters from homogenates, or compromise BBMV integrity.

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