STUDIES ON THE DEVELOPMENT of absorptive functions in the gastrointestinal tract have been carried out in a wide range of species (3, 15, 18, 22). Notwithstanding the valuable insights into nutrient transport provided by these studies, little information can be extrapolated to humans, because there are differences in intestinal structure and function, developmental trajectories, nutritional requirements, and feeding habits.

The present work examines the postnatal development of intestinal apical transport in the guinea pig, a species recognized as an interesting model for studying gastrointestinal physiology, because its timing in certain aspects of intestinal development closely resembles that of humans (9, 27).

The aim of this article is to characterize the developmental changes in brush-border uptake of a monosaccharide and an amino acid in the three regions of the small intestine (duodenum, jejunum, and ileum) in guinea pig at seven stages from the day after birth until adulthood. This period includes those stages when the synthesis in neonatal animals appears to be too slow to meet their needs (7).

 MATERIAL AND METHODS

Animals and Their Care

Male Dunkin Hartley guinea pigs (Cavia porcellus) were purchased from breeding colonies (Biocenter, St. Felu de Codines, Barcelona, Spain). Animals were housed in groups of one male, ten dams, and their litters in open cages under controlled humidity and temperature conditions. Controlled lighting provided a 12:12-h light-dark cycle. Litters remained with their mothers from birth until 2 wk of age, with constant ad libitum availability of water and solid diet. Guinea pigs were reared on a vitamin C-supplemented grass meal diet of FD1 Cobayos pellets (Interfauna Ibérica), green forage, and water. The commercial feed contained (g/kg diet) 183 crude protein, 36 lipid, 470 carbohydrate, 112 crude fiber, 74 ash, 11 calcium, 7.8 phosphorus, and 3.5 sodium and 2 mg/kg copper, 50,000 IU/kg vitamin A, 1,400 IU/kg vitamin D3, and 3,000 IU/kg vitamin C. The metabolizable energy content was 11.3 MJ/kg.

Sampling of Animals

Pregnant dams delivered throughout the day, so that the day after birth was designated as day 1. Experiments were performed in seven age groups of guinea pigs: 1-day-old and 1-, 2-, 3-, 4- to 6-, 8- and 9-, and 10- to 12-wk-old animals. All experiments were performed in the morning to minimize effects of any circadian rhythm and animals were not previously starved. Handling and killing of the animals were carried out in full accordance with the European Community guidelines for the care and management of laboratory animals. The small intestine was removed and trimmed from adherent mesenteric tissue. The gut was flushed and placed into chilled (2-4°C), oxygenated (95% O2, 5% CO2) Krebs-Henseleit solution.

Morphometrics

Body weight was recorded immediately before each experiment. Length of the small intestine, surface area, wet mass, and scraped mucosa were determined because rates of transport are sensitive to changes in amounts of tissue. To measure the length of the relaxed intestine from the pyloric sphincter to the ileocecal junction, the gut was carefully extracted, freed from adherent mesenteric tissue, and laid flat on a wet filter paper. The intestine was cut into three identical segments, flushed with 1 ml/cm of gut length chilled (2-4°C) saline.
solution, cut open lengthwise, and placed between two glass plates. The contours of the gently flattened gut were outlined immediately on transparent paper so that the surface area was measured by means of the IBAS image analysis program. Afterward, the intestine wet weight was recorded. Mucosa was scraped off the intestine with a glass slide to measure its weight. Both the mucosa and the remaining tissue were dried at 60°C for 24 h. Their dried weights were measured, and the proportion of scraped mucosa dry weight to total dry weight was calculated. Volumes (V) were calculated from measured parameters of length (L) and areas (A) according to the formula \( V = A^2/4 \times L \).

\( \alpha \)-MDG and L-Pro Uptake Measurements

Monosaccharide and amino acid total uptake were measured by the method described by Karasov and Diamond (10) using everted sleeves from at least three animals for experiment. Three regions of the small intestine were sampled: a proximal segment 10–12 cm long, starting from the pyloric sphincter, corresponding to duodenum; a segment 10–12 cm long, from the middle of the small intestine as jejunum; and a distal segment 10–12 cm long, terminating next to the ileocecal junction as ileum. Each segment was everted and cut into 15-mm-long sleeves, each of which was mounted onto a grooved metal rod of appropriate diameter (1.5–5 mm), and was tied to yield a snug fit. Until \( \alpha \)-MDG and L-Pro uptake were measured, the mounted sleeves were preincubated for 5 min in an incubation solution containing (in mmol/l) 95 ClNa, 6.2 CIK, 2.5 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, 7H2O, 24.9 NaHCO3, and 50 \( \alpha \)-mannitol. The osmolality of the incubation medium was 315 mosmol/l, the pH 7.4 when aerated with 95% \( \text{O}_2 \)-5% \( \text{CO}_2 \), and the temperature 37°C. Sleeves were incubated under the same conditions as described for the preparatory phase, but this solution also contained \( \alpha \)-MDG or L-Pro (both replacing an isosmotic amount of \( \alpha \)-mannitol) plus radioactive tracers. Furthermore, it was stirred at 1,200 rpm/min with a magnetic bar to reduce the effect of unstimred layers on uptake. Nutrient uptakes were estimated from the accumulation of radioactive solutes in the tissue. For that reason, the medium contained two labeled compounds: one tracer for the substrate (\( \alpha \)-[U-14C]MDG in sugar uptake studies and L-[U-14C]Pro in amino acid uptake studies); the other tracer was an extracellular space marker ([3H]polyethyleneglycol 4000) to measure the amount of substrate present in the adherent fluid but not actually absorbed. After incubations, the flat end of the rod was drained by touching it to the filter paper. The mounted tissue was cut off with a razor blade, and the surplus tissue outside the 1 cm length between the two grooves was cut away.

Afterward, the incubated sleeves were placed in a vial, their wet weight was recorded, and the substrate was extracted at 4°C with 0.1 mmol/l HNO3 overnight. An aliquot of the supernatant was added to Biogreen-1 cocktail from Sharlau (Barcelona, Spain), and the activities of [H] and [14C] were estimated with a scintillation counter. The optimal incubation period for studying \( \alpha \)-MDG and L-Pro uptake was determined by incubating the everted sleeves in 50 mmol/l of each substrate for 0.5, 1, 2, 4, and 8 min. In all experimental groups, uptake was linear for 2 min for \( \alpha \)-MDG and 1 min for L-Pro, indicating that in these periods the efflux of solute accumulated within the tissue was negligible. In accordance with these results, the time chosen for the experiments of nutrient uptake was 1 min for both substrates.

Expression of Nutrient Uptake

To compare small intestine uptake capacity to nutrient demands, uptakes were normalized to three measures of tissue quantity: sleeve wet weight, nominal surface area (cm²) (i.e., not taking into account the area amplification by villi and microvilli), and sleeve length (1 cm).

Chemicals

Radioisotopes \( \alpha \)-[U-14C]MDG and L-[U-14C]Pro and [3H]polyethyleneglycol 4000 were purchased from New England Research Products (Germany). All unlabeled reagents were obtained from Sigma, St. Louis, MO.

Statistics

Results were expressed as means ± SE of n guinea pigs. Unless otherwise stated, statistical differences between intestinal regions or age groups were established by ANOVA and Snedecor’s F test. However, kinetic parameters and ratios were compared by Student’s t-test, with the application of Bonferroni’s correction when more than two situations were compared. The P < 0.05 level was taken as significant.

RESULTS

Morphology

Body growth. The weight gain of Guinea pigs during the 1st wk of life is relatively slow: from 91 ± 9 g the day after birth to 117 ± 5 g on the 7th day. From that moment, the growth is steady, doubling the weight of the 1st day by the end of the 2nd wk (175 ± 6 g). At 10–12 wk of age, the body weight reaches 601 ± 13 g, which represents a 6.6 times increase of body mass from birth (Fig. 1A).

Intestinal growth. Intestinal weight, length, and surface area as a function of age increased according to the same pattern: from the day after birth until the 2nd wk, the growth rate showed a sharp rise, whereas from day 14 until the adult stage the increment was slower.

Intestinal weight augmented 4.2 times from the day after birth until adulthood. However, this growth rate was not linear throughout the period studied. The plot of small intestine mass as a function of age was fitted to two-segmented straight lines: one from birth until the 2nd wk and the other from this age until the 12th wk. The mass of the gut increased 2.6-fold, from 2.78 ± 0.31 g the day after birth to 7.22 ± 0.22 g at the 2nd wk (Fig. 1B). Beyond this age, the intestinal wet weight of guinea pigs continued to increase but at a slower rate. The animals increased intestinal weight by a factor of 1.6 from the 2nd wk until the 12th wk (11.6 ± 0.19 g).

Intestinal length growth rate was especially fast during the first 2 wk of life, increasing 1.6 times from the day after birth (70.8 ± 3.87 cm) until the 2nd wk of life (114.2 ± 1.81 cm) (Fig. 1C). After this age, the growth rate was slower, accounting for a factor of 1.4 from 2 wk up to 12 wk (156.8 ± 2.28 cm). The overall small intestine length increased by 2.2 times that of 1-day-old guinea pig.

Thus, as already mentioned for small intestine mass and length, surface area (Fig. 1D) increased disproportionately fast from the day after birth (36.7 ± 1.81 cm²) up to the 2nd wk of life (88.1 ± 2.81 cm²), representing a factor of 2.4. From the 2nd wk on, the increment in...
surface area was less marked, accounting for an average 147.4 ± 2.81 cm² at the 12th wk, 1.7 times the value at the 2nd wk.

The area-to-volume ratio was calculated as a function of age to estimate the amount of surface area available for absorption (Fig. 1E). This relationship was higher during the first 2 wk of life and was followed by a steady decrease until adulthood. This ratio provides additional evidence that guinea pigs increase the absorptive surface area so as to match the caloric necessities of the animal during the first 2 wk after birth.

The study of the optimal relationship between body mass and intestinal mass fitted two-segmented straight lines with different slopes, as in the model described by Konarzewsky et al. (12). The first segment showed a slope different from one and the second one did not differ significantly from zero. The interception of the first segment with the y-axis was not different from zero (Fig. 2). The intersection of the two segments was at body weight of 180 g, the weight of animals under 3 wk old.

When small intestine length and surface area were plotted against body mass, the data were fitted to two-segmented straight lines (Fig. 2, B and C) and followed the same trend as when the wet weight was represented as a function of body mass.

A linear positive correlation is observed when wet weight was plotted against length (y = 0.1x - 4.26; r = 0.927) and surface area (y = 0.078x + 0.16; r = 0.939). The slope calculated for wet weight against length was significantly higher than that of wet weight versus surface area.

Finally, the percentage scraped mucosa contributed to the whole thickness of the intestine was calculated. It remained constant up to the 3rd wk (76%), lowering to a value that kept constant thereafter (65% at the adult stage).

Nutrient Uptake

Time course of substrate uptake. On the basis of the measurements of nutrient transport as function of time, in all experimental groups uptake was linear for 2 min for α-MDG and 1 min for L-Pro (Fig. 3), indicating that in this period the efflux of solute accumulated within the tissue was negligible. In accordance with these results, the time chosen for the experiments of sugar and amino acid uptake at 50 mmol/l was 1 min. Total uptake of α-MDG. When uptake of α-MDG, measured at 50 mmol/l for 1 min, was expressed as a

Fig. 1. Changes in body weight (A), intestinal weight (B), length (C), surface area (D), and area-to-volume ratio (E) during development of the guinea pig. Individual values are represented, except for E, where results are expressed as means ± SE. Only SE that exceed size of symbol are shown. Regression lines were calculated by the least-square method. Equations and correlation coefficients (r) were A: y = 6.7x + 68.2 and r = 0.978; B: y = 0.34x + 2.19 and r = 0.837; C: y = 0.07x + 6.13 and r = 0.831; C: y = 3.34x + 2.19 and r = 0.862; y = 0.65x + 105.02 and r = 0.846; D: y = 3.97x + 29.74 and r = 0.866, y = 0.09x + 74.34 and r = 0.816; E: y = -0.20x + 8.03 and r = 0.982, y = -0.02x + 5.42 and r = 0.990.
function of tissue weight (nmol·mg⁻¹·min⁻¹) (Fig. 4), differences in apical fluxes with respect to intestinal regions or age groups were observed. In duodenum, uptake values reached a peak on the 1st day of life (11.11 ± 0.91 nmol·mg⁻¹·min⁻¹; n = 10), declining throughout the suckling period (6.86 ± 0.74 nmol·mg⁻¹·min⁻¹; n = 8), until weaning (4.79 ± 0.58 nmol·mg⁻¹·min⁻¹; n = 6) and remaining constant thereafter. In the jejunum and ileum, apical fluxes were maximal on the 1st day of life, decreasing thereafter. In the duodenum, the values remained constant until weaning (6.56 ± 0.44 nmol·mg⁻¹·min⁻¹; n = 13), and remained constant until the adult stage. The intestinal length rose less steeply with age than wet weight or surface area. α-MDG uptake was normalized to surface area (nmol·cm⁻²·min⁻¹) was higher during the 1st wk of life in duodenum than jejunum and ileum; from the 2nd wk on, the values remained constant. In ileum, the uptake reached a peak during the 1st day, keeping constant thereafter. From the day after birth until the 3rd wk, the apical uptakes were higher in duodenum and jejunum than in ileum. However, from that age on, no regional differences were found.

The intestinal length rose less steeply with age than wet weight or surface area. α-MDG uptake was normalized to weight of each region of the small intestine, and no significant differences were found during the period studied. Comparisons between regions yielded a similar pattern to those normalized to surface area. Total uptake of L-Pro. In the proximal small intestine, L-Pro uptake per milligram yielded a peak in the 1st wk (10.09 ± 0.50 nmol·mg⁻¹·min⁻¹; n = 12) (Fig. 5), decreased up to weaning (6.56 ± 0.44 nmol·mg⁻¹·min⁻¹; n = 13), and remained constant until the adult stage. Midintestinal L-Pro uptake was higher during the 1st wk of life (10.09 ± 1.19 nmol·mg⁻¹·min⁻¹; n = 6), followed by a progressive decrease until the 3rd wk (5.38 ± 0.30 nmol·mg⁻¹·min⁻¹; n = 7), and remained constant thereafter. In the distal small intestine, apical fluxes were higher during the 1st wk (9.05 ± 0.61 nmol·mg⁻¹·min⁻¹; n = 13), lessening to values that kept constant until 10–12 wk of age. At no age did duodenum, jejunum, and ileum differ significantly by Snedecor's F test in L-Pro uptake per milligram.

L-Pro uptake per square centimeter in duodenum showed a peak in the 1st wk and kept constant over the period studied. Patterns for L-Pro normalized to surface area in jejum, and ileum did not differ from the pattern for L-Pro uptake per milligram.

When L-Pro uptake was normalized to the length of the small intestine, a peak was reached in the 1st and 2nd wk in the proximal segment. However, in the mid and distal segments, L-Pro uptake per centimeter was uniformly distributed over the period studied.

Integrated nutrient uptake. Uptake capacity of the whole length of the small intestine was calculated (for both α-MDG and L-Pro at 50 mmol/l) by multiplying apical fluxes per centimeter and regional length for each segment and summing over the three regions. Integrated uptake for each nutrient increased with age. α-MDG uptake capacity normalized to body weight was higher on the 1st day of life, lessening with increasing age; L-Pro uptake normalized to body weight showed a peak at 1 wk, decreasing thereafter.

L-Pro and α-MDG uptake ratio. The L-Pro-to-α-MDG uptake ratio was calculated by dividing the total fluxes of both substrates in each region and age group. In 1-day-old animals, duodenum was better adapted to transporting monosaccharides than amino acids, be-
cause the L-Pro-to-α-MDG ratio was 0.76 ± 0.07 (n = 10), expressed as means ± SE (Fig. 6). However, L-Pro-to-MDG ratio reached a peak of 1.47 ± 0.09 (n = 8) in the 1st wk, decreasing until the 3rd wk (1.16 ± 0.1; n = 6), and remaining constant thereafter. Jejunum was better adapted to the uptake of amino acids than to sugars during the first fortnight, showing a maximum in the 1st wk (1.54 ± 0.14; n = 6). From the 3rd wk on, the L-Pro-to-α-MDG ratio did not differ significantly from one. Ileum maintained a higher ratio than the other regions at all stages of development. Maximal values were shown in 1-wk-old guinea pigs (2.11 ± 0.14; n = 10).

DISCUSSION

Research on intestinal transport has been mostly focused on the mechanisms of nutrient absorption in altricial species, in which the timing of certain aspects of gastrointestinal development is quite different from those in the human (2, 4, 21). Altricial mammals express transporters toward the end of gestation (3, 23), whereas in the intestine of humans and guinea pigs, transporters are already expressed at the beginning of the second half of gestation (2, 6). The gastrointestinal tracts of altricial species are far from mature at term and undergo considerably more development during the neonatal period and at weaning, when abrupt changes in gastrointestinal structure and function (4, 14, 21). Conversely, in humans and guinea pigs, the major phases of gastrointestinal development occur in utero without marked changes in mucosal structure and function during the postnatal period (11, 26).

Guinea pig, a precocial mammal, was chosen as an experimental animal in which to study nutrient transport, as it may more closely reflect the development of human infant gastrointestinal tract (9, 27). At birth, the guinea pig is little dependent on maternal milk for either nutritional or nonnutritional purposes and can ingest solid food soon after delivery (24, 25).

In the present work, the developmental and regional changes in the uptake of α-MDG and L-Pro were studied from birth to adulthood. A study of the intestinal macroscopic morphology was also carried out, because the way nutrient uptake is normalized influences the interpretation of the results.

Morphology

The studies on macroscopic morphological parameters are displayed in Fig. 1. The body weight results (Fig. 1A) followed the same pattern as those obtained by Buddington and Diamond (5) in cats, which also have nutrient transporters at birth. During the 1st wk, cat intestine undergoes no significant increase in length, area, and mass, in contrast to guinea pig, which shows
In the growing guinea pig, a marked increase was observed in the absorptive surface area (Fig. 1D) through a lengthening of the gut. When the slopes from length and surface area during the first 2 wk of life were compared, no significant differences were found. This increase enables the intestine to process food in a shorter time without any sacrifice of extraction efficiency.

The ratio of surface area to volume is biologically important and interesting in morphometric terms. By dividing the area of an intestinal region by its volume, the area (cm²) per unit of volume (ml) can give an estimate of the amount of surface area available for absorption (19). Figure 1E shows that during the 1st
wk of life higher values were observed, which indicates an advantageous surface area-to-volume relationship, so that absorption is enhanced. Snipes and Kriete (19) applied this relationship to several species, although only at one age, and they found that the rabbit was the animal that had the same basal area-to-volume ratio as guinea pig.

The growing guinea pig undergoes variations in growth rate that are influenced by environmental and physiological factors. Konarzewski et al. (12) developed a mathematical model in birds that described the optimal allocation of energy to the growth of the digestive tract and the rest of the body; our purpose was to check this model in mammals. The mass of the guinea pig small intestine was plotted against the body mass (Fig. 2) did not fit a straight line when plotted in a double logarithmic scale, as Diamond’s group found for other animal species (18). Rapid intestinal growth was observed in the guinea pig during the first 2 wk after birth, followed by slower growth thereafter.

The fastest intestinal growth rate in guinea pig has been found during the first 2 wk after birth, thus enabling efficient entry of nutrient resulting in adequate development of the animal (13).

Nutrient Uptake

In guinea pig, as in other species, the most relevant changes in nutrient uptake occur during the first days after birth (4, 8, 18, 20, 22). In guinea pig, already at birth uptake rates per milligram of tissue showed a peak for α-MDG in the three segments studied. For the amino acid L-Pro, initial fluxes were near their highest values in duodenum and at their maximal values in jejenum and ileum. The results obtained are consistent with the pattern exhibited by other species that express transporters prenatally such as pig (18), cat (5), and rabbit (4). However, rats display two peaks on uptake, one during the suckling phase and another after weaning (21).

The highest nutrient uptake rates observed at birth in most mammals are consistent with the relatively large quantity of energy and nutrients needed to meet the high metabolic requirements in developing animals (2).

During the postnatal development of guinea pig, α-MDG uptake in duodenum per milligram of tissue declined from the day after birth to the 2nd wk. From that period on, no statistically significant differences were found until the adult stage. In jejenum and ileum, the uptake observed the 1st day after birth diminished at the 1st wk to values that kept constant thereafter.

On the other hand, the initial fluxes of L-Pro were higher during the 1st wk. From the 2nd wk on, the uptake remained constant until the adult stage.

In accordance with the macroscopic morphological results, the decline in the uptake expressed per milligram cannot be attributed to a decrease in the percentage of intestinal mucosa, because the proportion of mucosa remained virtually constant up to the 1st wk (76%) followed by a decline to a value that kept constant until the adult stage (65%).

The higher values observed at birth or soon afterward when initial fluxes were expressed per milligram of intestinal tissue might be explained in that neonatal intestine nutrient uptake occurs along the whole crypt-villus axis and neonatal enterocytes have a long life span (3). After this period, the subsequent lifelong crypt-villus gradient of transport activity is established, being maximal at the villus tip and minimal or absent in the crypts. Weaver and Carrick (23) observed a 40% decline in villus height during the first 2 wk of life and a 25% increase in crypt depth during the first 4 wk of postnatal life. This “dilution” of transporting cells may explain the observed tendency for uptake per milligram of tissue to decline with age (8).

The regional study was carried out because previous works using other animal species have shown changes in nutrient uptake along the small intestine (1, 7, 20). In guinea pig, the regional study indicated a decline in the α-MDG per milligram of tissue from the proximal to the distal region of the small intestine at the day of birth, from which age the α-MDG uptake was evenly distributed throughout the length of the gut. On the other hand, L-Pro was equally distributed in all the age groups studied.

In guinea pig, the ratio of amino acid to sugar uptake capacity (L-Pro to α-MDG) changes with increasing age in each segment studied. One of the factors that may affect the L-Pro-to-α-MDG uptake ratio is the composition of the diet (4). During the period studied, the animals undergo a shift from milk to adult diet. The composition of guinea pig milk has a protein and a carbohydrate content of 8.1 and 4.3%, respectively,
which means an amino acid-to-carbohydrate ratio higher than in the adult diet (26). This could be reflected by the higher L-Pro-to-\(\alpha\)-MDG ratio in the duodenum and the jejunum during the first fortnight.

The higher values of L-Pro-to-\(\alpha\)-MDG ratio observed in the ileum until the 3rd wk of life indicate that this segment is the best adapted to transporting L-Pro, suggesting a scavenger role for the distal part of the small intestine to retrieve amino acids.

The present data give further support to the hypothesis that nutrient transporters change ontogenetically to satisfy the functional demands imposed by development. Shifts in dietary composition alone cannot be used to predict changes in the intestinal nutrient transport function during development and a genetic program should be involved.

Perspectives

In the present study, a decrease in the activity of the intestinal monosaccharide and amino acid transport was found during the postnatal development of the guinea pig. The age-related changes in macroscopic morphology have been established from the day after birth to the adult stage. Although the present study characterizes the influx of \(\alpha\)-MDG and L-Pro throughout development in guinea pig, a species of recognizable interest due to its similarities to humans, several questions remain unsolved. The kinetic constants of the mediated and nonmediated transport of monosaccharides and amino acids are of interest due to their similarities to humans, several questions remain unsolved. The kinetic constants of the mediated and nonmediated transport of amniv and L-Pro should be established to know the mechanisms responsible for regional changes during development. The molecular mechanisms involved in regulating the activity and expression of the transporters need to be studied to find causes for the notable decline in nutrient uptake during the first hours after birth. To this end, the use of specific antibodies and cDNA may provide a better understanding of the regulation of these transporters throughout development.

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