Anatomical Gut Growth and Development

Jacques Schmitz

Department of Pediatrics, Hôpital des Enfants Malades, 149, rue de Sèvres, 75743 Paris Cedex 15, France

The anatomical development of the human gut has been extensively studied and is now well understood even at the ultrastructural level. However, the mechanisms controlling gut development remain obscure. Although tissue interactions (1) and endocrine factors (2) play a major role, it is clear from isografts of fetal gut in adult animals that genetic programming is essential (3).

In this chapter I shall give a concise description of gut development in the human fetus, and present relevant examples from among the sparse recent data on the molecular genetics of gut development. At present these concern only the mouse.

ORGANOGENESIS

The primitive gut is formed during the fourth week of intrauterine life by invagination of the dorsal part of the yolk sac. The epithelium of the gut and associated glands develops from endoderm, while the connective tissue and muscular elements derive from mesoderm. From the fifth week of gestation up to term the intestine lengthens 1000-fold (4). This dramatic increase in length, together with the rapid development of the liver, results in a temporary herniation of the primitive intestinal loops into the umbilical cord, which starts at the sixth week of gestation. From the 10th to the 12th week the intestinal reenters the abdominal cavity from its cranial end and carries on a 270° counterclockwise rotation. At 11 to 13 weeks of gestation, the small intestine measures between 25 and 40 cm in length, at the 20th week approximately 1 m, 1.70 m between the 27th and the 35th week, and from 2 to 2.5 m at term, while the colon lengthens from 25 to 50 cm during the same period (5).

MORPHOGENESIS OF VILLI AND CRYPTS

It is during the period of herniation that the villi begin to differentiate. Villus formation starts in the duodenum at about the eighth week, and, proceeding distally, reaches the jejunum at 9 to 10 weeks and the ileum at 11 to 12 weeks of gestation. By the 14th week, scanning electron microscopy shows that the villi have acquired
ANATOMICAL GUT GROWTH AND DEVELOPMENT

their typical finger-shaped aspect in the proximal intestine (6,7). The epithelium is multilayered up to the 10th week. At 8 weeks, it is made of two to four layers of undifferentiated columnar cells, with short irregular cytoplasmic projections bordering the lumen, small intracytoplasmic deposits of glycogen, and frequent mitotic figures suggesting a high proliferative capacity (8), which is confirmed by the scatter of $^3$H-thymidine uptake in nuclei throughout the stratified epithelium (9). Indeed, the rapidly proliferating epithelium soon completely fills the duodenal lumen, which is again patent by the 9th to 10th week. Despite similar proliferative activity, total occlusion of the gut lumen does not occur distal to the duodenum (4,6). Crypt development also proceeds distally. The first crypts appear in the proximal intestine at 10 to 11 weeks of gestation as downward buddings of the epithelium into the mesenchyme, between the new villi. They are present in the ileum and the colon 1 to 2 weeks later (4). This early appearance of villi and crypts in the human contrasts markedly with intestinal development in other species such as the rat, where villi appear late in gestation and crypts appear well after birth (6,10).

As villi form, the epithelium becomes columnar and by 12 weeks villi are lined by a single layer of epithelial cells. In the proximal intestine, between the sixth and ninth week of gestation, vacuoles develop within the stratified epithelium. These resemble "secondary lumina" in that the epithelial cells that delineate the vacuoles form well-defined microvilli on their luminal plasma membranes and are joined by continuous tight junctional strands (8). The precise role of these "secondary lumina" in the transition from stratified to single-layered epithelium is as yet not fully understood in the human. In the rat they expand and contribute to the formation of short villi that are lined by a simple columnar epithelium (10). It is suggested that in the human fetal intestine these vacuoles are part of the same process (6).

CELLULAR DIFFERENTIATION

Overlapping with the formation of the crypt-villus axis, distinct cell types differentiate.

Crypt Cells

Undifferentiated crypt cells appear as crypts form in both the proximal and the distal intestine. They have large basal nuclei, abundant free ribosomes, little formed endoplasmic reticulum (ER), and, in contrast to undifferentiated crypt cells in adults, prominent glycogen deposits and practically no lysosomes (8).

Paneth cells appear in the crypts during the 12th to 14th week of gestation. They rapidly acquire long stands of rough ER (RER) and well-developed Golgi complexes. Secretory granules with electron-dense cores are highly heterogeneous, both in size and number. As the fetus develops, the secretory granules become more homogeneous, as in the adult, and are restricted to the apical cytoplasm (8).

Glandular extensions leading to acini deep in the lamina propria and resembling
Brunner's glands are seen in the proximal intestine at 14 to 15 weeks of gestation. The columnar cells forming the glandular acini have a less well organized RER and smaller secretory granules than corresponding cells in the adult (8).

**Epithelial Absorptive Cells**

Epithelial absorptive cells represent by far the most important class of cells in the epithelium, and their ultrastructure has been extensively studied (11-13). From 6 to 8 weeks of gestation, immature cells lining the lumen present occasional cytoplasmic projections, but no true microvilli (11,12). However, by the 8th to 10th postfertilization week, absorptive cells show numerous, well-ordered microvilli at their apical poles, averaging 1.0 μm length and 0.2 μm in width (their mature dimensions), and their central core is made of filaments that are variable in number but of consistent diameter (3.5-5 nm) (11). Simultaneously, brush-border cytoskeleton proteins, the calmodulin binding proteins (110 kDa protein, caldesmon, fodrin), at first (8th week) uniformly present throughout the epithelium, become localized to the apical part of the epithelial cells (12th week) (14). At 8 weeks, desmosomes are already present but junctional complexes are absent. These appear by the 10th week and the cell membranes beneath the developing terminal web are interdigitated (13).

At the same time, glycogen accumulates in large supranuclear and infranuclear vacuoles. Irregular electron-dense lysosomal elements, also termed "meconium corpuscles," appear around the 12th week of gestation in the supranuclear cytoplasm (12,13). By the 14th week of gestation, proximal and distal enterocytes are morphologically similar, with a well-organized terminal web and a brush border that shows its typical aspect both by transmission and scanning electron microscopy (7,13). At that time an apical tubular system and "meconium corpuscles" are prominent features of the absorptive cell (13). Between 15 and 17 weeks, both cell substructures show a marked development in the distal but not the proximal intestine, followed by a progressive decrease from the 18th to the 22th week of gestation, after which they may be undetectable (13,15). Both systems are suppose to be involved in the uptake of luminal macromolecules (15,16). Finally, during the fourth month of gestation a reduction in glycogen content occurs, with further development of the smooth endoplasmic reticulum. Thus the absorptive cell is morphologically not different from its adult counterpart during at least the last trimester of gestation. This is at variance with what is seen in laboratory animals, particularly the rat, where a mature brush border is not seen before birth (10).

**Endocrine Cells**

Several ultrastructural (12,17) and immunohistochemical (18) studies have shown endocrine cells to be present in the fetal intestine by the 10th to 12th week of gestation. Primitive and precursor endocrine cells, characterized by their pale cytoplasm, with
secretory granules that are denser and smaller in the primitive cells than in the precursor cells, have already appeared in the stratified epithelium of the proximal intestine at 9 to 10 weeks of gestation (17). During the following 2 weeks "transitional" cells appear, characterized by the simultaneous presence of secretory granules of the precursor type (large—1 μm in diameter—and pale) and of the types found in one of the four specific adult endocrine cells (EC, S, I, G). These "transitional" cells are rarely seen after the 16th to 18th week of gestation. Finally, mature-looking S cells (secreting secretin) with granules of 100 to 200 nm diameter, EC cells (secreting motilin, serotonin, and substance P) with dense granules of 250 to 380 nm diameter, I cells (secreting cholecystokinin) with granules of 220 to 280 nm diameter, and G cells (secreting gastrin) with granules of variable densities and of 250 to 480 nm diameter are present in the proximal intestine between the 9th and 12th week of gestation, and approximately 1 week later they are present in the distal intestine (17). S, EC, and I cells are found only in the crypts. Cells secreting somatostatin (D cells) and GIP (D1 cells) appear during the same period. Enteroglucagon-secreting cells (L cells), unlike the other fetal endocrine cells, appear in the distal small intestine before (10th to 11th week) appearing in its proximal part (13th week of gestation) (17). It is likely that "primitive," "precursor," and "transitional" cells represent immature precursors of adult endocrine cells. These fetal cells synthesize and store adult-like, and probably active, peptides since most have also been characterized immunologically (18).

Other Specialized Cells

Goblet cells differentiate as early as endocrine cells, being recognizable within the stratified epithelium of 9- to 10-week-old fetuses (8,19). Tuft, or caveolated cells, characterized by bundles of dense filaments extending from the microvillous cores deep into the supranuclear region and by apical multivesicular bodies, are first noted at 16 weeks of gestation in the villi. Their role is still unknown (8). M cells, known to be involved in the transfer of macromolecules from the lumen to the cells of the underlaying lymphoid follicles, are first noted at 17 to 18 weeks of gestation in the distal epithelium, overlaying aggregated lymphocytes. Characteristic microfolds are already present along their luminal plasma membrane and membrane-bound vesicles are abundant in their apical cytoplasm (8).

BIOCHEMICAL MATURATION OF THE SMALL INTESTINAL SURFACE

Although the small intestinal surface appears morphologically mature well before birth in the human, recent biochemical studies of the brush-border membrane have shown subtle differences in the fetus compared to the adult. This has been particularly well demonstrated for a group of integral brush-border proteins, the hydrolitic enzymes (20). These differences are probably related to differences in glycosylation, as suggested by studies of the interactions of these enzymes with lectins (21). Such modifications of glycosylation during postnatal development have been described in rabbits (22) and rats (23) where they are probably linked to developmental changes
in glycosyltransferase activity (24). Increased sensitivity and response to Escherichia coli heat-stable enterotoxin in young rats (25), increased cholera toxin binding to rabbit and rat newborn brush-border membranes (26,27) compared with adult animals, as well as the appearance of receptor activity for enteropathogenic E. coli RDEC-I in rabbit (28), might be linked to these modifications of glycosylation. Such age-related differences in sensitivity for E. coli heat-stable enterotoxin have also been demonstrated in children, where the number of receptors for the toxin rapidly decreases with age, as does the degree to which it stimulates guanylate cyclase (29).

Experiments in laboratory animals suggest that not only brush-border membrane proteins and mucus but also the lipid phase of the membrane undergo biochemical changes during development. Such changes include higher lipid-to-protein and lower cholesterol-to-phospholipid ratios (30), leading to an increased membrane fluidity in newborn compared to adult animals (31). Such developmental differences may directly influence the maturation of enterocyte transport functions (32) and antigen uptake (16). Although similar data are still lacking, such changes in the composition of the intestinal surface most probably also occur in humans, though their importance remains to be established.

GENETIC PROGRAMMING

Genetic programming of gut development is most clearly demonstrated when fetal isografts are transplanted in adult animals; without any luminal or endocrine factors, enzyme activities mature (33) and cells migrate and home normally (3) with the expected proximal-distal gradient. Experiments in transgenic mice have recently shed some light on the genetic mechanisms determining small intestinal development.

Homeobox genes are known to play a critical role in controlling the cephalocaudal organization of Drosophila. Similar genes (Hox genes) are expressed along the cephalocaudal axis of the developing mouse. Transcripts from nine of these genes have been identified in the developing and/or adult mouse intestine (34). However, the demonstration that expression of the genes can affect development processes was obtained only recently in transgenic mice overexpressing the Hox-1.4 homeogene. After in situ hybridization of transcripts in 12-day-old embryos, labeling restricted to the mesenchymal layer of the developing gut was twice as intense as in wild-type embryos. Interestingly, this overtranscription of Hox-1.4 mRNA correlated with the later development of a condition resembling congenital megacolon. Thus provided the first example of a mutant phenotype affecting gut development associated with the expression of a mammalian homeobox-containing gene (35).

Transgenic mice have also been fruitfully used to gain insight into the mechanisms leading to organ-specific, region-specific, and even developmental stage-specific gene expression (36). By fusing the promoter region of rat liver (L) or intestinal (I) fatty acid–binding proteins (FABP) to a reporter, the human growth hormone (hGH) gene minus its regulatory elements, it has been possible elegantly to determine, for example, the role of the 5′-non-transcribed region of the I-FABP gene in the region-specific expression of hGH. Nucleotides −1.178 to +28 limit expression of the reporter to the intestine with the proximal-distal gradient expected for the I-FABP
gene; however, nucleotides -277 to +28 lead to a 250-fold decrease in expression of hGH in the ileum, indicating the loss of critical upstream CIS-acting sequences (37). Similar experiments are currently providing important clues to the spatial differentiation of the intestinal epithelium (3).

The study of the genetic programming of gut development is just beginning. The coming years will reveal its major role in the control of the exceedingly intricate processes leading to a mature small intestine in the newborn.

REFERENCES


**DISCUSSION**

**Dr. Guesry:** You have given us a lot of information about the development of digestive capacity. What I have been unable to discover, and wonder whether you have any information about, is the time at which the gut starts to be able to handle elemental diets. When can the intestine begin to absorb amino acids, glucose, and medium-chain triglycerides?

**Dr. Schmitz:** There are no functional studies in fetuses of, say, 15 or 20 weeks' gestation. There is, however, evidence that glucose-linked sodium absorption is already operational by 12 weeks of age, and that amino acids can be absorbed by 10 to 12 weeks. It seems likely that the gut could be used as an organ of absorption by the middle of the second trimester, though nobody has tried this yet.

**Dr. De Curtis:** What effects do steroids given to the mother before birth have on gut development?

**Dr. Schmitz:** There are few data relating to humans. Bile acid synthesis is increased by steroids, resulting in a larger bile acid pool at birth.
Dr. Verellen: Nature has presented us with an interesting problem of gut development in duodenal atresia. It is striking how tiny the gut looks in these cases. Is it known what happens to the intestine after reconstruction? What are the capabilities of such intestines? Could the amniotic fluid play a role in determining the genetic potential of the duodenal cells?

Dr. Schmitz: We don't know much about the causation of duodenal atresia. Some atresias are clearly familial and probably linked to a mutation somewhere. As to the functional capacity of the gut after the atretic segment is removed, it seems that in most cases the gut has become hypoplastic because of disturbed blood supply, so it is often very difficult to reverse the situation. The hypoplasia is a secondary consequence of the atresia, and probably not directly linked with it. Nothing is known about the length of the intestine in such cases.

Dr. Swyer: A practical point for this discussion is, when can we start to feed premature infants? Many things have to come together to allow feeding to take place, not only anatomical development but also motility, processing, transfer, and so on. It seems to me that one of the important prerequisites is the actual introduction of foodstuffs into the intestine, because these probably trigger the production of essential enzymes.

Dr. Schmitz: I agree to some extent that the introduction of foodstuffs is important, but it has only a short-term effect in bringing about the maturation of enzymes; it does not change the whole pattern. It was shown in the 1970s, for example, that the blood glucose curve in premature infants after a glucose or a lactose load was similar to the curve in term infants after the third day of feeding. Feeding caused a 3-day advantage but not more than that.

Dr. Swyer: However, there have been studies showing that the development of the gut in terms of weight and length is strongly dependent on oral feeding.

Dr. Sedaghatian: We know that the lungs of small-for-dates babies mature earlier than normal and they have less risk of developing hyaline membrane disease. These babies often pass meconium in the absence of signs of fetal hypoxia. Do such babies have early maturation of the intestine?

Dr. Salle: The SGA baby is a stressed baby and produces high levels of corticoids. It is likely that these will mature the intestine.

Dr. Priolisi: It was shown some years ago that small-for-gestation infants are unable to digest and absorb proteins adequately during their first few days of life (1). This seems to contradict the view that maturation of the gut has occurred in these infants.

Dr. Salle: I don't think this is a reflection of digestive capacity but rather of motility. Gut motility is severely deranged by asphyxia.

Dr. Micheli: We all know about bronchopulmonary dysplasia. Is there an analogous condition in the gut—gastrointestinal dysplasia?

Dr. Schmitz: I doubt whether such a condition exists. The anatomical development of the gut is very much in advance of that of the lungs.

Dr. Simmer: Many very premature babies develop necrotizing enterocolitis. Isn't this a sign that the gut is not ready?

Dr. Schmitz: I was referring to the maturation of the molecular mechanisms of digestion and absorption by the gut rather than to the development of motility and vascularization. These are certainly not mature and well organized in very premature babies, you are right.

REFERENCE