

Gastrointestinal Development: Implications for Infant Feeding

Josef Neu, MD

Martha Douglas-Escobar, MD

The field of gastrointestinal (GI) development has been advancing at a rapid pace in the past 30 years.¹ The level of GI maturity of an individual infant is a major determinant of whether the infant will be able to meet nutritional needs by sole use of the GI tract or if parenteral means will be necessary. The GI tract is not only an organ for digestion and absorption of nutrients; it also performs major endocrine, neural, and immunologic functions. In this chapter, anatomic, functional, and biochemical development will be described and related to means by which enteral nutrition can be used to optimize health and prevent disease. The focus of this chapter will be on neonatal period.

ANATOMIC DEVELOPMENT

The intestine undergoes tremendous growth during fetal life. It elongates 1,000-fold from 5 to 40 weeks. The length doubles in the last 15 weeks of gestation, reaching a mean length at birth of 275 cm. In the small intestine, finger-like projections, the villi, are already formed at 16 weeks of gestation. In the large intestine, villi are also present, but these partially regress at around 29 weeks of gestation. Microvilli begin to cover the apical surface of the small intestinal epithelium so that by adulthood, the intestinal surface provides the largest interface between the outside environment and the internal milieu (approximately 2,000,000 cm², which is about the size of a tennis court).

There are numerous cell types in the small intestine. These include the intestinal absorptive epithelium, Paneth cells (involved in secretion of defensins and other peptides implicated in the innate immunity), Goblet cells (involved in the secretion of the mucous lining the intestine), and other cell types involved in the intestinal neuroendocrine and immune systems. After intestinal epithelial cells undergo mitotic division in the crypt, they migrate up the villus, where they undergo differentiation and become actively absorbing cells and then after apoptosis become sloughed into the intestinal lumen. There is only one population of cells that migrate deeper into the crypt and these are the Paneth cells. Specific issues regarding these cell types will be discussed later in this chapter.

DEVELOPMENT OF MECHANICAL FUNCTION

Readiness to feed by mouth is a major developmental milestone for the premature neonate. Mechanical function of the GI tract, the major determinant of this readiness, includes suck–swallow coordination, gastroesophageal sphincter tone, gastric emptying, and intestinal motility. These will be discussed separately even though they function along a continuum in the GI tract to propel food to areas where digestive absorptive functions occur.

Suck–Swallow Coordination

Very premature infants prior to about 32 weeks' gestation have a suck–swallow pattern that differs from that of the more term infant.² The very preterm infant is not able to coordinate sucking activities during the swallowing process. Furthermore, preterm infants swallow preferentially at different phases of respiration than those of their full-term counterparts² and this results in an inefficient and potentially dangerous pattern that may result in aspiration of gastric contents into the trachea and lungs if these infants are fed by mouth. Thus, most of the very low birth weight (VLBW) infants are tube fed. Feeding directly into the stomach and/or intestine by gastric and transpyloric feeding, respectively, bypasses the immature suck–swallow coordination in these infants, but remains subject to other immaturities such as gastroesophageal reflux (GER), poor gastric emptying, and immature small intestinal motility. When transitioning to nipple feeding with a bottle, use of nipples that offer relatively high resistance to flow may be helpful in preventing a rate of intake higher than the infant's immature suck–swallow respiration pattern can manage.

Gastroesophageal Junction and Gastroesophageal Reflux

Ganglia are present in the esophagus at 5 weeks' gestation and complete their migration to the rectum by 24 weeks' gestation. Despite this, the esophagus of the premature infant demonstrates slower propagation velocities and contraction duration that is more prolonged than in the older child. At about 28 weeks' gestation, the lower esophageal sphincter resting pressure is only 4 mmHg, but it increases to

adult values (18 mmHg) by term.³ Dramatic increases in GER have been seen after diaper changes, an intervention that results in increased intra-abdominal pressure.⁴ Although GER occurs commonly in premature infants, as do chronic lung disease, apnea, and bradycardia, it remains unclear whether a cause–effect relationship exists between these problems, and is a highly debated issue. The prevalence of actual gastroesophageal reflux disease is still also a debatable issue. At this juncture, most of the techniques to determine reflux have relied on acid measurement techniques, but it is known that reflux does not need to be acid to cause reflex apnea. Since apnea is a major problem encountered in the neonatal intensive care unit (NICU), if a cause–effect relationship exists and is present in a significant number of infants, pharmacologic (antireflux medications) or feeding technique–related (thickening, positioning, type of nipple) therapies could have a major impact on treatment and/or prevention of apnea and bradycardia. Studies employing newer techniques of measuring reflux based on nonacid measurements, for example, impedance, may yield important new information in this area. Although the short-term problems associated with GER remain an area of major interest, the recent suggestion that GER in low birth-weight infants may play a role in the development of adenocarcinoma of the esophagus when these children reach late adulthood is intriguing.⁵

Gastric Emptying

Gastric emptying is slower in premature than in term infants^{6,7} and leads to a greater residual volume of gastric contents; thus, it is closely tied to the problem of GER. Infants between 32 and 39 weeks' gestation provided with progressive increments of caloric density decreased the rate of gastric emptying.⁸ Changes in osmolality from 279 to 448 mOsm/kg do not significantly alter the rates of gastric emptying of isocaloric formulas.⁸ These are potentially intriguing findings in that the fetus normally swallows significant amounts of low-caloric-density amniotic fluid (up to about 450 mL/d) during the last trimester of pregnancy,⁹ but has difficulty tolerating higher caloric density formula and even breast milk postnatally when born prematurely. In adults, the rate of gastric emptying is controlled by feedback from the small and large intestine. Stimulation of duodenal

receptors by acid, fat, carbohydrates, tryptophan, or increasing osmolality decreases rates of gastric emptying.⁸ Very little information on the ability of duodenal feedback to control the rate of gastric emptying in the VLBW infants between 25 and 32 weeks' gestation is available. However, providing a formulation that is low in some of the agents (high carbohydrate, lipid, etc) that slow gastric emptying may be one technique to improve tolerance in terms of gastric emptying and also not to overwhelm the more distal intestinal tract motility and digestive absorptive functions. Several studies suggest that therapeutic agents that increase gastric emptying rates in children and adults are also effective in premature infants. Randomized trials of erythromycin are mixed but some suggest improvement in feeding tolerance without significant complications.^{10–12} Metoclopramide also increases gastric emptying rate in preterm and term infants¹³ but it is somewhat difficult to monitor side effects (extrapyramidal movement disorders or tardive dyskinesias) of this agent in this age group. Cisapride, in one small trial, did not increase gastric emptying rates in preterm infants.¹⁴ However the efficacy and safety of this agent would need to be studied in larger randomized trials. Cisapride is currently not available in the US market because of concerns related to cardiac arrhythmias.¹⁵ Nasojejunal (transpyloric) feeding bypasses the immature gastric emptying and is often employed to decrease the likelihood of reflux-related aspiration and apnea, but there are little data to support the efficacy of this technique in decreasing morbidity.

Intestinal Motility

The small bowel motility patterns are poorly developed before 28 weeks' gestation. Gastroanal transit ranges from 8 to 96 hours in premature infants as compared with 4 to 12 hours in adults. Berseth et al¹⁶ in studies of small intestine showed disorganized motility patterns between 27 and 30 weeks' gestation, which progress to a more mature pattern so that migrating myoelectric complexes are present at 33 to 34 weeks' gestation. In preterm infants, the motilin receptor is not present until 32 weeks' gestation, and the cyclic release of motilin is not present. Despite this, several of the studies of the motilin agonist erythromycin^{11,12} have demonstrated improved feeding tolerance. Whether erythromycin might act through mechanisms not related to motilin agonists remains speculative. Minimal enteral feedings also increase motility and this will be discussed later in this chapter.

DEVELOPMENT OF DIGESTIVE–ABSORPTIVE FUNCTION

Protein Digestion

Our knowledge of digestive–absorptive processes has increased significantly in the past three decades. Hydrolysis of protein begins in the acid environment of the stomach and proceeds distally in the intestine through the action of various proteases.

Gastric acid secretion is limited in VLBW infants. In the first 24 to 48 hours after birth, intragastric pH remains at about 5.5 to 7 (whereas the gastric pH in normal children and adults is 2 to 3) and is relatively resistant to pentagastrin. However, both basal and pentagastrin-stimulated acid secretion doubles from the first to fourth week of postnatal life in preterm infants.¹⁷ Gastric acid serves as a barrier to microorganisms. This is an important consideration when using H₂ blockers because decrease in gastric acid production may lead to a higher load of bacteria in the more distal regions of the intestine.¹⁸ Several studies have now supported that critically ill patients treated with H₂ blockers have a higher incidence of nosocomial sepsis¹⁹ and one recent study suggests a greater incidence of necrotizing enterocolitis (NEC) with the use of H₂ blockers.²⁰

The pancreatic protease digestive cascade is catalyzed by food-stimulated secretion of enterokinase from the upper small intestinal epithelium. Enterokinase catalyzes the activation of trypsinogen to trypsin, which in turn activates several other inactive zymogens into proteases. Active luminal enterokinase is detectable at 24 weeks' gestation but its activity level is relatively low and reaches only 25% adult activity at term.²¹ This may limit protein digestion and may be responsible for an increased capability of larger antigens and/or microorganisms to pass into the intestine without breakdown by luminal enzymes. After luminal digestion, small peptides and amino acids are absorbed and transported to the villus capillaries. The absorptive proteases are usually present and fully active before 24 weeks' gestation and should not be limiting.²² This would suggest that hydrolyzed protein would be better tolerated. Indeed, one study demonstrated more rapid establishment of full enteral feeding with a hydrolyzed protein formula compared to a nonhydrolyzed protein formula.²³ Nevertheless, these studies did not utilize a human milk–fed control group. In other studies, human milk was much better tolerated than formulas.²⁴ Before initiating use of a hydrolyzed formula for the premature it is important to remember that many hydrolyzed protein formulas are not nutritionally formulated for the premature infant.

Lipid Digestion and Absorption

Lipid makes up about 50% of the nonprotein energy content of human milk and formulas. However, both term and preterm infants have pancreatic insufficiency when compared to older children and adults and this may result in malabsorption of certain types of lipids.

The digestion of lipid can be split into phases.²⁵ The luminal phase of lipid digestion involves lipase deesterification of triglycerides to 3-monoglycerides and free fatty acids, and bile acid–mediated micellar solubilization. Several lipases participate in the fat digestion, including the lipase found in human milk (bile salt–stimulated lipase), lingual, gastric, pancreatic, and epithelial lipases. Human milk has a lipase (absent in cow's

milk and formulas) that becomes active in the small intestine lumen only in the presence of bile acids.²⁵ Lingual lipase is secreted by glands at the base of the tongue and is involved in gastric lipid hydrolysis. Other lipases involved in lipid hydrolysis are produced in the stomach; others are secreted from the pancreas. Bile acids synthesized in the liver are critical to efficient fat digestion and absorption. VLBW infants have a lower duodenal concentration of bile acids due to lower synthesis and ileal reabsorption of bile.^{26,27} Therefore a lower micellar solubilization leads to lower lipid absorption. Long-chain fatty acids but not medium-chain fatty acids depend on bile acids for solubilization and thus are the most susceptible to inefficient absorption. Despite this limitation, meta-analysis of studies comparing medium-chain triglycerides (MCTs) to long-chain triglycerides feeding do not show clear benefits in terms of weight gain.²⁸

The next phases, lipid absorption and assimilation, include the permeation of fatty acids and 2-monoglycerides from the lumen into the cell, intracellular reesterification, chylomicron formation, and transport to the chylomicrons from the cell into the circulation. After luminal digestion, fatty acids and monoglycerides approximate the absorptive surface, and enter the intracellular milieu by processes that are not yet well understood. After entry into the cell, MCTs undergo a relatively simple process of assimilation where they do not undergo reesterification and chylomicron formation, as do the long-chain lipids. MCTs are taken directly into the portal venous system whereas chylomicrons formed from long-chain fats enter the lymphatics. Table 1 summarizes some²⁹ of these differences. In conditions where the lymphatics are obstructed (eg, chylous ascites), MCT-predominant formula is thus recommended (rather than long-chain triglyceride-predominant formula).

Lipid requirements are limited²⁹ to the 18-carbon essential fatty acids (linoleic or linolenic acid). However, there is considerable interest and debate about the capability of neonates, especially prematures, to convert the dietary essential fatty acids (linoleic and linolenic) into longer chain fatty acids (with 20 or more carbons) using desaturase and elongase enzymatic pathways. The end

Table 1 Comparison of Short-, Medium-, and Long-Chain Fatty Acid Digestion and Assimilation

Overview of Fatty Acid Uptake

- Short- and medium-chain fatty acids
 - Enter portal blood directly from enterocytes
 - Bound to albumin in blood as Albumin–Free Fatty Acids (FFA) complex
 - Oxidized in liver or elongated and used for triglyceride formation
- Long-chain fatty acids
 - Form chylomicrons in intestinal cell
 - Drain into the lymphatics via the lacteal
 - Enter bloodstream at the thoracic duct upstream from liver
 - Slow entry into the blood

result is the formation of the long-chain polyunsaturated fatty acids (LCPUFAs),³⁰ which are critical in the formation of eicosanoids and structural components of the central nervous system. Preformed LCPUFAs are found in relatively high concentrations in human milk but are not found in commercial intravenous lipid preparations or, until recently, most neonatal formulas. A meta-analysis and systematic review of data from premature and term infants indicate advantages of docosahexaenoic acid (DHA, an omega-3-LCPUFA)-supplemented formulas over unsupplemented formulas in both behavioral- and electrophysiological-based measurements of visual acuity.^{31,32} Although there is a suggestion from recent research that there may be some benefit to VLBW infants, whether addition of these to formulas results in improvement in cognitive neurodevelopment remains the subject of current investigation.³⁰ Nevertheless, while on minimal enteral feeding and/or parenteral nutrition (PN), premature infants receive very little, if any, preformed omega-3-LCPUFAs, a deficiency of which is likely to have detrimental consequences in both nervous tissue formation and inflammatory responses.

Most of the lipids from the formulas or intravenous lipids are derived from vegetable oil (rich in omega-6- but not omega-3-LCPUFAs). Therefore, the majority of essential fatty acids provided to neonates are derived from the omega-6 family (linoleic acid). Numerous studies have demonstrated that products derived from the omega-6 fatty acids (2-series prostaglandins) have a greater inflammatory effect than those derived from the omega-3 fatty acids (3-series prostaglandins).³³ Although there is a considerable amount of information in adults about the omega-3 fatty acids ameliorating inflammation, that is, rheumatoid arthritis, coronary artery disease, and other conditions, very little information exists in human neonates. One study in a rat model of NEC suggests a decrease in NEC with omega-3 fatty acid supplementation.³⁴ Whether providing more omega-3 lipids would decrease NEC, chronic lung disease, or neurological problems related to inflammation in human infants remain intriguing possibilities that require additional investigation.

CARBOHYDRATE DIGESTION AND ABSORPTION

Carbohydrates ingested by neonates are either natural lactose (the primary carbohydrate in most human and mammalian milks) or in the form of glucose polymers, sucrose, or hydrolyzed starches. Pancreatic amylase, which cleaves internal one to four glucose bonds to maltose, maltotriose, limit dextrin, and glucose, is a major enzyme that hydrolyzes starches. Pancreatic secretion is poorly developed in the first several months of life; therefore, at least this mode of starch hydrolysis could serve as a limiting factor and leave a lot of undigested starch in the intestine. Experimental studies suggest that glucose polymers (18 to 29 glucose units) can be hydrolyzed by salivary

amylases, but this digestion still falls substantially short of that accomplished by usual concentrations of pancreatic amylase.³⁵ Many infant formulas including those used for preterm infants contain corn syrup solids or tapioca, which are partially hydrolyzed starches. The more extensively the starch is hydrolyzed, the less reliance is placed on an immature digestive capability, but the greater the osmolality (and risk of hyperosmolar diarrhea and/or intestinal inflammation). Maturation of mechanisms for carbohydrate absorption occurs in a defined sequence during human fetal development.^{22,29,36,37} The intestinal epithelial absorptive enzymes, lactase, sucrase, maltase, isomaltase, and glucoamylase are at mature levels in the term newborn.²¹ In the preterm infant, sucrase, maltase, and isomaltase are usually near fully active, but lactase activity, which increases markedly from 24 to 40 weeks, may be low depending upon gestational age.²¹ Despite these developmental patterns, clinical lactose intolerance is uncommon. Postnatal adaptive responses to ingested carbohydrates lead to competent carbohydrate utilization.³⁸ Furthermore, there is a colonic salvage of nonabsorbed carbohydrates. The colonic microbiota ferment the nonabsorbed carbohydrates to hydrogen gas and short-chain fatty acids (SCFAs, which are easily absorbed).³⁶

Feeding and composition of the feeding has been shown to affect the development of intestinal lactase activity.³⁸ Preterm infants who received early enteral feedings had greater lactase activity at 10 and 28 days of age (by 100 and 60%, respectively) than a control group that had feedings initiated later (standard time of initiation of feedings). At 10 days of age lactase activity was also greater in human milk- versus formula-fed infants. The time required achieving full enteral feedings, the number of abnormal abdominal X-ray examinations, and the total numbers of abdominal X-ray examinations were inversely related to lactase activity. It was concluded that early feeding increases intestinal lactase activity in preterm infants. Lactase activity is a marker of intestinal maturity and may influence clinical outcomes.

The presence of a high lactose concentration in human milk thus should not be a contraindication for its use in the VLBW infant. Feedings for VLBW infants are rarely initiated at levels intended to meet the infants' entire nutritional requirements and are usually advanced slowly. The rationale for using a lactose-free formula instead of human milk or even a commercial lactose-containing formula is weak and may theoretically be harmful. Slow initiation of enteral feedings is unlikely to exceed the lactose hydrolytic and salvage capability of the small and large intestine. This is especially unlikely when the quantity fed is less than 50% of the caloric requirement provided via the GI tract (unless the infant's bowel has been radically shortened by surgery). Human milk also contains several lactose-derived oligosaccharides and other glycoconjugates that may play an important immunologic role.

Studies examining the crypt to villus gradient of intestinal carbohydrase activities demonstrate that the majority of lactase activity is found at the mid-to-upper villus, whereas sucrase, maltase, and glucoamylase are concentrated at the midvillus region.^{39,40} This is likely to be pertinent to intestinal injury and villus damage. Lactase is usually the first enzyme to be lost and the last to be fully regenerated after injury. Congenital lactase deficiency is extremely rare. A large proportion of the world's population develops lactose intolerance in the first few years of life because of a decline in lactase activity, but further discussion of this phenomenon and its clinical implications is beyond the scope of this chapter.

INNATE DEFENSE AND BARRIER FUNCTION DEVELOPMENT

The GI tract presents the largest surface area of the body to which antigens and microbes are exposed. The intestine must therefore have intricate mechanisms to allow entry of nutrients and other beneficial molecules while preventing potentially harmful microbes and other agents from gaining entry into the inner milieu. Shortly after birth, the establishment of an intestinal bacterial community is very important for maintenance of normal homeostasis. The VLBW infant in the NICU is presented with special challenges. Several factors lead to a hostile GI environment that predisposes the infant to disease. These include the introduction of feeding tubes into the stomach or more distal intestine, the routine use of broad-spectrum antibiotics that select for resistant pathogens that thrive in the unusual microbial environment of the NICU, intrinsic immaturities of the infant GI tract, and the lack of adequate enteral nutrition. Thus, combining total parenteral nutrition (TPN), the lack of enteral nutrition, and the introduction of a hostile microbiota into an immature GI tract offers a fertile environment for initiation of various forms of morbidity related to intestine-derived systemic inflammation and perhaps even programming of pathologic immune responses that leads to lifelong consequences.

Intestinal defenses may be broadly divided into innate and adaptive categories. The importance of the innate system is that it constitutes the first line of host defense. Each of the components of the innate system acts in concert to maintain mucosal integrity in the intestine. Several of these components are presented here.

Breast Milk

Breast milk is an integral component of innate defense for the neonate because numerous components of breast milk are known to be protective to the infant. These include immunoglobulins, lactoferrin, lysozyme, glycoconjugates, oligosaccharides, and various types of white blood cells. Antibodies in human milk reflect the antigenic repertoire of the mother's intestine and respiratory tract. Colonization of the lactating mother's

intestine with specific strains of *Escherichia coli* results in the appearance of corresponding antibodies in the mother's milk.⁴¹ This effect is achieved by seeding-primed plasma cells to the breast tissue. Thus the repertoire of antibacterial, antiviral, antifungal, and antitoxin antibodies in human milk is considerable. It varies from mother to mother and is determined by the antigens ingested and inhaled by her infant.⁴² Thus, if the same bacteria colonize the mother and infant, and if there is close contact, the mother can produce specific antibodies that can protect the infant through her milk. This comprises the so-called enteromammary system.⁴³ Furthermore, mother's milk is colonized by bacteria that may play a positive role and act as a natural "probiotic."⁴⁴ Oligosaccharides in mother's milk may act as nutrients for beneficial commensal bacteria (a "prebiotic" role) and fermentation of these oligosaccharides and lactose in mother's milk may lead to the production of SCFAs that play a "postbiotic" role, especially butyrate, which can be effective as a major fuel for colonocytes, an anti-apoptotic, proproliferative agent, that may also aid in the strengthening of intercellular tight junctions,⁴⁵ and also stimulate the synthesis of glucagon-like-peptide 2, a hormone that is highly trophic for the intestine.⁴⁶ Human milk contains high levels of interleukin 10 (IL-10) and transforming growth factor beta, which are anti-inflammatory and serve to modulate otherwise uncontrolled propagation of intestinal inflammation.⁴⁷

Gastric Acid and Pancreaticobiliary Secretions

Gastric acid and pancreaticobiliary secretions decrease the load of viable microorganisms as well as intact dietary protein antigens that reach the small intestine.⁴⁸ Pancreatic insufficiency can last through the first year of life.⁴⁹ Lack of stimulation of gastric acid and pancreaticobiliary secretions can adversely affect the intestine by allowing a greater bacterial and/or antigenic load to pass to the distal intestine. Proteolysis will destroy the structure of antigens, thereby destroying the epitopes for immunologic recognition.⁵⁰ Studies by Walker et al⁵¹ demonstrated an increase in bovine serum albumin in the intestine of rats given bicarbonate at the same time they were fed with bovine serum albumin. This is important because many VLBW infants are not provided nutrients by the enteral route for the first several days of life, thereby having minimal intestinal protease and acid stimulation, with resultant high gastric pH. These same infants are often given H₂-blocking agents routinely often for poorly defined reasons in order to decrease gastric acid secretion. The breakdown of intact protein molecules, bacteria, and other microbes may therefore be less. The detrimental effect of this practice is evidenced by the greater incidence of hospital-acquired sepsis and even NEC seen in infants who receive H₂-blocking agents.^{20,52} Studies in newborn rats have also demonstrated that use of H₂ blockers leads to greater bacterial translocation.¹⁸

SURFACE EPITHELIUM

Intestinal Absorptive Epithelium

The absorptive lining epithelium of the primitive intestine is initially stratified but becomes a single layer of columnar cells at the end of the first trimester. The intestinal epithelial enterocyte derives from the same undifferentiated stem cell that gives rise to columnar absorptive epithelium, mucus-producing goblet cells, and Paneth and M cells in the intestine.⁵³ The function of these cells beyond nutrient absorption is well recognized and some of their major roles include bacterial mucosal cross-talk and innate barrier function.

Goblet Cells

These are specialized mucus-secretory cells found throughout the intestine. Goblet cells mature from the base of the crypt (proliferative zone) and migrate along the crypt-villus axis. After 5 to 7 days they reach the tip of the villi and are exfoliated (by a particular programmed cell death called "anoikis"). They secrete mucus/mucin, which is very important in the nonspecific protection of the gut lining. Intestinal mucus is a complex gel that covers the surface of the villous epithelium and contributes significantly to cytoprotection at the same time that it offers many ecological advantages for the microbiota. It is composed primarily of water and electrolytes but also contains mucins, glycoproteins, immunoglobulins, glycolipids, and albumin.⁵⁴ Bioactive factors such as lactoferrin, lysozyme, and glycolipids from breast milk may also be contained within the surface mucus gel.⁵⁵ Mucus is secreted into the lumen of the intestine from storage vacuoles in goblet cells that are interspersed among enterocytes along the villus-crypt axis. Mucous gels containing foreign dietary antigens and pathogenic bacteria are sufficiently stable to keep their contents trapped. The intestine is capable of responding to a threatening insult by the rapid outpouring of mucin and at the same time, resident microbiota can induce an increase in the production of mucins. The mechanism of this response is not well understood. Mucins may be classified according to their structure as gel forming, soluble, and membrane bound. Mucins develop binding sites for lectins, selectins, adhesion molecules, growth factors, cytokines, and chemokines, suggesting that they are implicated in cellular signaling. Mucins lower the diffusion of large molecules (which limit the antigenic load exposure) but allow the passage of small nutrient molecules.

Marked changes in their composition occur during development. Mucin from small intestine of newborn rats contains more protein than does the adult rat intestine.⁵⁶ Carbohydrate composition also changes; the newborn has less fucose and *N*-acetyl-cysteine than does the mucin from adults. Levels of mucin in the small intestine decrease in experimental protein-calorie undernutrition, which could reduce the GI protection during the perinatal period.⁵⁷ Lower amounts of

mucin caused by malnutrition could affect the normal protective function of mucin, particularly in the perinatal period. Certain nutrients such as threonine have a major effect on mucin production⁵⁸ and need to be considered in the nutrition of critically ill individuals.

Paneth Cells

Paneth cells represent one of the four major epithelial cell lineages in the small intestine and are the only lineages that migrate downward into the crypt base after originating in the crypt stem cell region. The location of Paneth cells adjacent to crypt stem cells house suggests that they play a critical role in defending epithelial cell renewal. In response to pathogen attack, the Paneth cells secrete a wide spectrum of antimicrobial peptides (AMPs) against gram-negative and gram-positive bacteria, fungi, protozoa, and viruses. The AMPs are able to kill the pathogens by different mechanisms. They are able to attach to and insert into microbial membrane bilayers to form pores, reduce the synthesis of cell wall and nucleic acid, and inhibit enzymatic activity. Some of the major enteric pathogens have developed resistances to AMPs as a way to evade innate mucosal defenses.

One of the main group of AMPs secreted by Paneth cells are alpha-defensins, also known as cryptdins.⁵⁹ These peptides have hydrophobic and positively charged domains that can interact with phospholipids in cell membranes. This structure allows defensins to form pores that disrupt bacterial membrane function, leading to cell killing. Paneth cells are stimulated to secrete defensins when exposed to bacteria (both gram-positive and gram-negative types) or other bacterial products such as bacterial products as lipopolysaccharide (LPS). Alteration of AMPs released from the Paneth cell is detrimental to host defense against *E. coli* infection in the neonatal small intestine. In addition to defensins, Paneth cells secrete lysozyme and phospholipase A₂, both of which have clear antimicrobial activity. This battery of secretory molecules gives Paneth cells a potent arsenal against a broad spectrum of agents, including bacteria, fungi, and even some enveloped viruses.

M Cells

M cells in the intestine do not have well-developed microvilli and allow macromolecular transport, whereas the absorptive enterocyte has better developed microvilli. M cells occur only in the follicle-associated epithelia that overlie organized mucosa-associated lymphoid tissues.⁶⁰ They are specialized for delivering foreign antigens and microorganisms to organized lymphoid tissues within the mucosa of the small and large intestines. They are thought to play a major role in specific immunity to luminal antigens. Their relationship to disease in the human neonate remains to be established.

Interepithelial Junctions

Special mechanisms are required to allow passage of certain substances between intestinal epithelial

cells and prevention of passage by other substance. Special junctions and interdigitations join the intestinal epithelial cells as seen in Figure 1A and B. The tight junction (zonula occludens) is the most apical component of the junctional complex and serves as the permeability barrier between the external and internal milieus of the body. The tight junction completely encircles the apical end of absorptive cells as a belt-like band. Several transmembrane proteins and proteins on the cytosolic leaflet are a part of the tight junction. These include the claudins and occludin protein families. Interactions of some of these proteins with the actin cytoskeleton are a major determinant of tight junction structure and play a role in the regulation of tight junction assembly.⁶¹ Just below the tight junction, the adhesion junction (zonula adherens) is connected to actin filaments in the cytoplasm and anchors each cell to adjacent cells. Below this, the desmosome (macula adherens) functions in a similar manner to the zonula adherens. The relationship between density of zonula occludens strands and epithelial resistance has been demonstrated in cell culture studies.⁶² These junctions allow for physiologic passage of fluids, electrolytes, and small macromolecules up to 11 amino acids long,⁶³ but larger proteins may not pass this route under physiologic conditions. However, pathologic insults may alter these junctions so that they allow abnormal passage of particles. Additionally, these intercellular junctions are exquisitely sensitive to

modulation by small molecules. SCFAs produced by bacterial fermentation such as butyrate have been shown to enhance restoration of mucosal barrier function after injury.⁶⁴ Administration of glutamine, an amino acid known to be conditionally essential during critical illness, can also prevent the disruption of tight junctions and subsequent increased paracellular permeability induced by a variety of insults.^{65,66} Our ability to manipulate these junctions may be key for prevention of several diseases that are autoimmune in nature and may at least partially relate to “a leaky gut” in its pathogenesis.⁶⁷ Figure 2 illustrates some of these diseases.

Epithelial Turnover and Restitution

It is unclear whether the infant can respond to injury to the same extent as the adult. In nonhuman mammals, the intestinal epithelial turnover in the infant is much slower (4 to 5 days) in the infant than in the adult (2 days).⁶⁹ If the turnover in human neonates is longer than the adult as in other mammals, this may be significant in that regeneration of injured mucosa in the infant will be much slower than the adult. In addition, environmental factors may contribute to poor restitution in intestinal epithelial cells after injury. In an animal model of NEC, it was determined that intestinal restitution was significantly impaired compared to control animals.⁷⁰ The mechanism of this effect was evaluated in vitro with rat crypt cells (IEC-6), which were shown to undergo

significant inhibition of restitution after exposure to LPS.⁷⁰ Glutamine has been demonstrated to prevent cytokine-mediated apoptosis in human colonic cells.⁷¹ Another amino acid, arginine, also induced intestinal epithelial migration, but through the mammalian target of rapamycin (mTOR) pathway.⁷² The role of these amino acids in counteracting intestinal stresses and insults deserve further investigation.

THE INTESTINAL ECOSYSTEM AND MICROBIOTA

It is clear that there is a close interplay between the major cell types lining the intestine, nutrients, and the microbiota, which are all constituents of the intestinal ecosystem. Reduction of normal commensal bacteria in the context of infection or after antibiotic treatment may interfere with nutrient availability and impair beneficial stimulation of the GI immune response. Similarly, a lack of enteral nutrients for both the host and the microbiota may also result in detrimental consequences.

We are beginning to appreciate the profound changes that occur in the intestinal ecosystem when young mammals are weaned from their mother’s milk. An example of such a change that normally occurs is in the angiogenins, potent AMPs released from the Paneth cells of the crypt.⁷³ In mice raised in a conventional environment, angiogenin mRNA expression is markedly

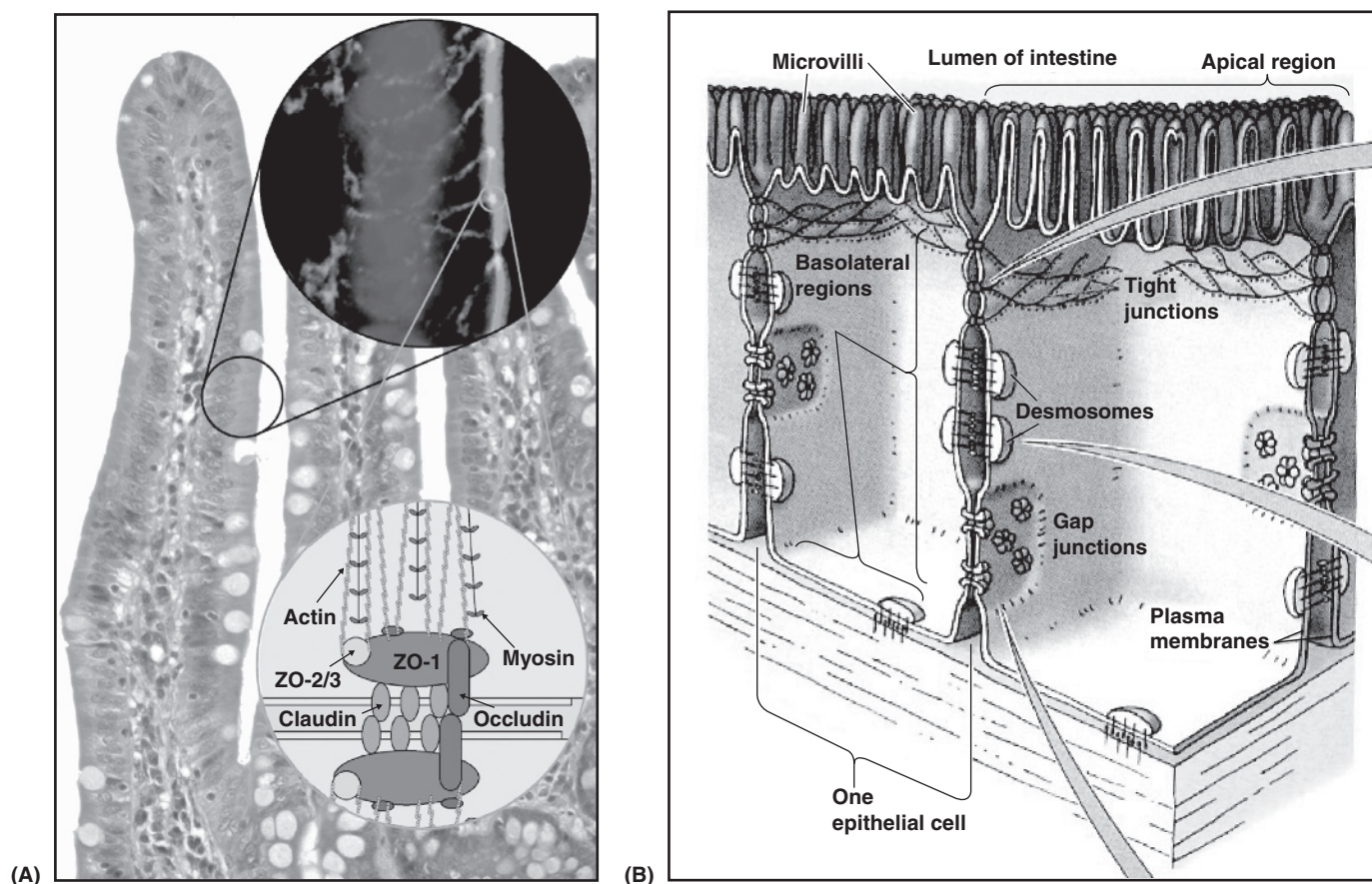


Figure 1 (A) Intestinal villi with immunofluorescence of tight junction proteins. (With permission from reference 68.) (B) Relation of tight junctions to the other junctional complexes.

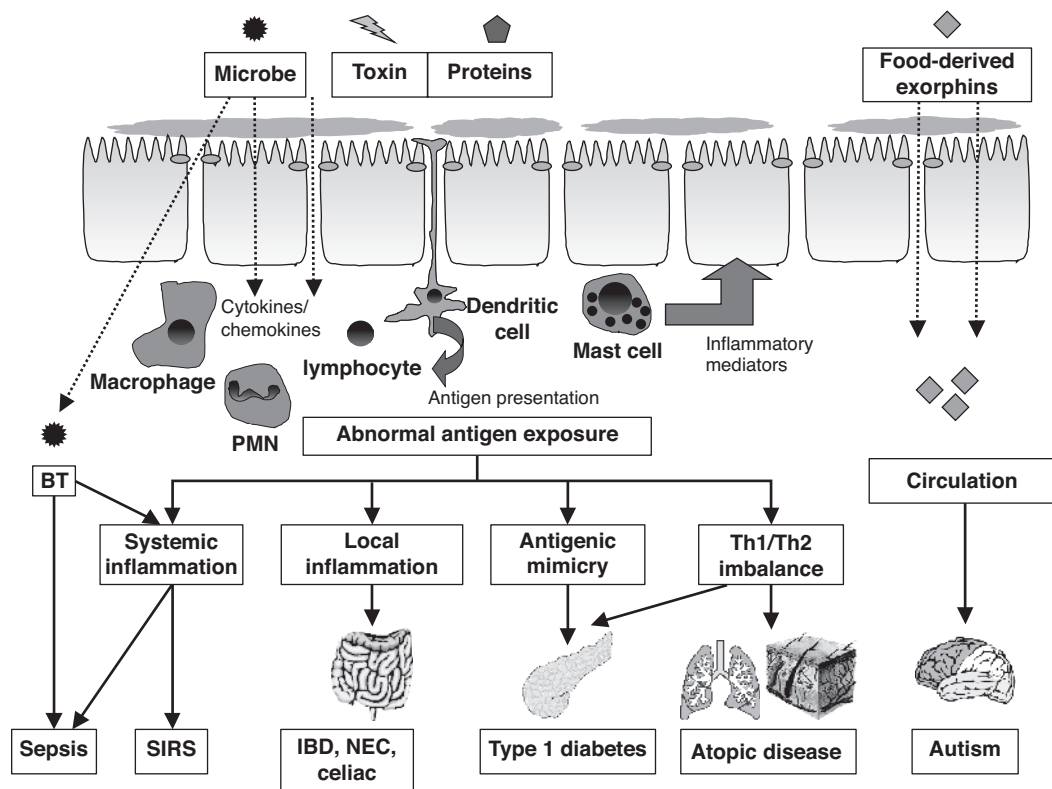


Figure 2 Problems associated with a “leaky gut.” (Reprinted with permission from reference 67.)

increased at the time of weaning from mother’s milk to an adult diet. This change occurs to a much lesser degree in germ-free mice.⁷⁴ It is also thought that commensal bacteria, through “cross-talk,” and the production of angiogenins are critical for normal villus capillary development. One dramatic example of this is seen in the lag in development of the villus capillaries in the mouse small intestine grown in a germ-free environment versus normal growth in an environment containing normal mixed flora or even only one species of commensal bacterium (*Bacteroides thetaio-tamicron*).⁷⁵

Most of the premature infants in NICUs are treated with broad-spectrum antibiotics. The effect of this practice on the resident intestinal microbiota is not known but could have potentially detrimental effects. Studies in rodents have shown marked alterations in the ability of the intestine to tolerate otherwise benign insults when the intestine is depleted of microbes^{76,77} and other studies have shown marked alterations in genes responsible for intestinal development after treatment with only one antibiotic.⁷⁸ Several studies in the past few years support the notion that providing certain bacteria as a functional food (probiotics) may be beneficial in the prevention of diseases such as NEC.

DEVELOPMENT, INFLAMMATION, AND NUTRITION

The reliance on PN while providing few or no enteral nutrients may be highly detrimental because it promotes intestinal inflammation, atrophy, and sepsis, as seen in Figure 3. The role

of enteral nutrients in the prevention of the intestine-derived inflammatory response syndrome is becoming increasingly recognized.⁷⁹ Certain individual nutrients may play an important role in modulating these effects. For example, glutamine depletion in LPS-treated Caco-2 (an adult transformed cell line) and, even more dramatically, in H4 (human fetal cells), results in marked upregulation of IL-8 production.⁸⁰ Similarly, in infant rats fed a commercial diet by gastrostomy and stimulated with LPS, glutamine decreases chemokine-induced neutrophil chemoattractant (the rodent counterpart of human IL-8) production in the intestine.⁸¹ Several studies have suggested similar effects in vitro for probiotics, but studies in neonatal animals showing regulation of intestinal inflammation by probiotics have only recently been published.⁸² Studies in human intestinal epithelial cell cultures have also shown that human milk suppresses IL-1 beta-induced IL-8 production.⁸³ Many of these nutritional agents appear to be acting through modulation of nuclear factor kappa B signaling and transcriptional regulation, but precise mechanisms are yet to be defined.

INTERVENTIONAL APPROACHES TO NEONATAL ENTERAL FEEDING

Total Parenteral Nutrition versus Minimal Enteral Nutrition versus Trophic Feeding

It is becoming increasingly recognized that in addition to immaturity of the neonatal intestine, a lack of enteral nutrients when receiving TPN contributes to mucosal breakdown, bacterial

translocation, and an increased propensity to systemic inflammation⁷⁹ (see Figure 3). The preterm, especially the VLBW preterm, infant frequently is also highly stressed because of respiratory insufficiency, thermoregulation, and other challenges. Protein requirements are frequently not met in these infants. The trend in the past decade has been to provide greater amino acids by the parenteral route⁸⁴ in the acute phase of illness in the first weeks after birth and then to transition to human milk or formula by the enteral route. Although a step in the right direction for improved nutrition, this is likely to be suboptimal: If these infants receive any enteral nutrition at all in the first weeks of life, it is usually in the form of “minimal enteral nutrition.” Despite often being termed “trophic nutrition” it is unlikely that minimal enteral (roughly defined as an amount of nutrition that does not meet the nutritional requirements for growth but confers some benefit, and is usually less than 20% of the total nutritional requirements provided for at least 5 days) along with PN is adequate to provide a significant trophic effect on the intestine or whether it actually enhances barrier function.⁸⁵ However, it is clear that the use of TPN is significantly associated with hospital-acquired sepsis,⁸⁶ which is increasingly thought to have its origin in translocation of intestinal bacteria⁸⁷ and intestinal inflammation. Studies in animals support that provision of enteral nutrients can reverse the effects of PN-associated translocation of intestinal bacteria as well as the exquisite proinflammatory potential of the intestines.⁸⁸ Although the recent trends toward early increases in intravenous protein intake may provide benefits in terms of protein synthesis, decreased catabolism, and perhaps even somatic growth, there are questions whether this practice provides benefits for maintenance of the GI barrier function and optimal growth of the GI tract, which is a prerequisite for modulation of several disease processes where intestine-mediated breakdown and systemic inflammation play a role. These include NEC, hepatic inflammation, chronic lung disease, and progression of periventricular leukomalacia.^{89–91} There is a great need for optimization of an enteral formulation

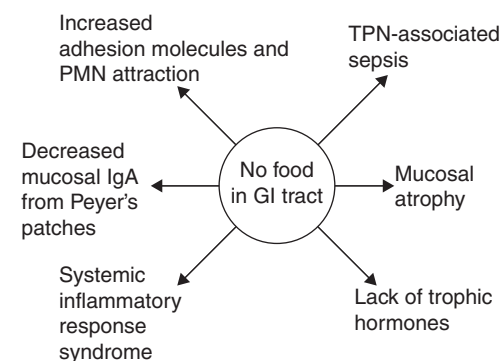


Figure 3 Problems associated with total parenteral nutrition.

that can be safely used in critically ill infants as an adjunct to minimal enteral nutrition shortly after birth that will decrease the need for PN.

Minimal Enteral Nutrition

Considerable emphasis has been placed on nutrition of the low-birth-weight infant after the period of critical illness. Until recently there were very few systematic studies on how to best nourish the infant during the period of critical illness while on mechanical ventilation and when the infant is likely to be the most catabolic in the first 2 to 3 weeks of life. This is a period of extremely high vulnerability and time of high nutrient requirement. A lack of essential nutrients during this time may result in significant short-term morbidity as well as life-long consequences.

When and how quickly should we advance enteral feedings? Because of individual characteristics of each patient, one feeding protocol or guideline cannot be used for all infants. From the available studies, minimal enteral feedings should be instituted within the first days of life. There is no clear evidence that the concomitant use of umbilical catheters, continuous positive airway pressure (CPAP), mechanical ventilation, indomethacin, or the presence of apneic and bradycardic episodes preclude the use of minimal enteral nutrition because of an increased risk of NEC.

When we decide on feeding strategies for premature infants, it is important to remember that infants given early minimal enteral nutrition have faster maturation of motor patterns and release of GI hormones than infants given no enteral feedings. Early fed infants have less feeding intolerance, establish oral feedings sooner, and do not differ in their incidence of NEC. In addition to timing of the feedings, the manner in which the feeding is provided may also be important.⁹² Motor responses appear to be less intense on the more diluted formulas. The amount of volume does not appear to have a benefit in maturing motor function. Motor responses are equally intense whether feedings are instilled intragastrically or transpylorically. They are similar whether feedings are given chilled, at room temperature, or warmed to body temperature. When infants are fed a slow infusion over 120 minutes, they display an intense fed response that is accompanied by brisk gastric emptying. However, when the same volume is fed over 15 minutes, duodenal motor responses are far less intense and are accompanied by delayed gastric emptying. Because two-thirds of all preterm infants display an immature duodenal fed pattern that is accompanied by delayed gastric emptying, many preterm infants may not be as physiologically prepared to process bolus feedings as well as slow infusion feedings from the viewpoint of motor activity. A suggested scheme for initiation of minimal enteral feedings in VLBW infants is illustrated in Figure 4.

Some Future Directions

The fetus receives about 450 mL/d of amniotic fluid through the GI tract during the last trimester of pregnancy,⁹ whereas the most enteral volume

supplied to premature infants during the first weeks of life is what has been termed “minimal enteral nutrition,” and usually constitutes approximately 5 to 10% of the volume that would go through the intestine per day in utero. This suggests that the postnatal immature GI tract actually might be able to tolerate more volume than what is commonly provided, but in a different form than the nutrient-dense preparations currently provided. Compositional differences such as osmolality and lipid composition, which act as stimuli to decrease motility, are likely to slow gastric emptying and intestinal motility, critical factors in tolerance to enteral feeding.

There are several individual nutrients that could be utilized to enhance intestinal growth and function. Here, we will discuss a few of them, enteral protein, glutamine (GLN), long-chain omega-3 PUFAs and SCFAs.

Protein

Since protein is the major macronutrient required for growth, it is likely that protein (amino acids) provided via the parenteral versus the enteral route does *not* provide the intestine with an equivalent amount of trophic stimulation for intestinal growth. In a study using an infant canine model, it was demonstrated that approximately 40% of the intake required for normal growth needs to be supplied by the enteral route before a trophic effect on the mucosa occurs.^{85,93} This intestinal trophism is most likely derived from protein rather than lipid or carbohydrate. Mucosal growth and prevention of atrophy are critical because they relate to several functional capabilities including digestion and absorption, bacterial, or antigenic translocation and the capability for luminal microorganisms and/or toxins to incite an inflammatory response causing pathology such as NEC or systemic inflammation.

Glutamine

Most NICUs do not routinely supplement critically ill premature babies with the amount of GLN they would be receiving had they remained in utero. A comprehensive review of GLN action is beyond the scope of this chapter. A significant period of enteral nutrition *and* GLN deprivation experienced by the majority of VLBW infants is a likely contributor to the lack of intestinal mucosal integrity and exquisite susceptibility to intestinal injury.

Despite several studies over the past decade that demonstrate efficacy and safety of GLN supplementation in highly stressed, critically ill patients' babies born prematurely receive very little, if any, GLN during this highly vulnerable period because they rarely reach full enteral feedings (which contain GLN) for weeks and because GLN is absent in routinely used intravenous solutions. Early studies of enteral GLN supplementation in VLBW infants demonstrated a decrease in hospital-acquired sepsis along with a decrease in the stimulation of CD16 and HLA DR markers on peripheral blood lymphocyte populations, consistent with decreased stimulation of the inflammatory response secondary to decreased translocation of proinflammatory antigens.⁹⁴ A recently completed multicenter trial of enteral GLN supplementation in 649 VLBW infants showed significant improvement in intestinal function and a decrease in Grades 3 and 4 intraventricular hemorrhages or periventricular leukomalacia in survivors.⁹⁵ An additional, more recent study, using enteral GLN supplementation, showed a beneficial effect on hospital-acquired sepsis.⁹⁶ One large study of intravenous GLN supplementation in neonates, however, did not show benefit.⁹⁷ Better targeting of patient subgroups, dosages, route, and timing of administration will be necessary to fully evaluate the presence

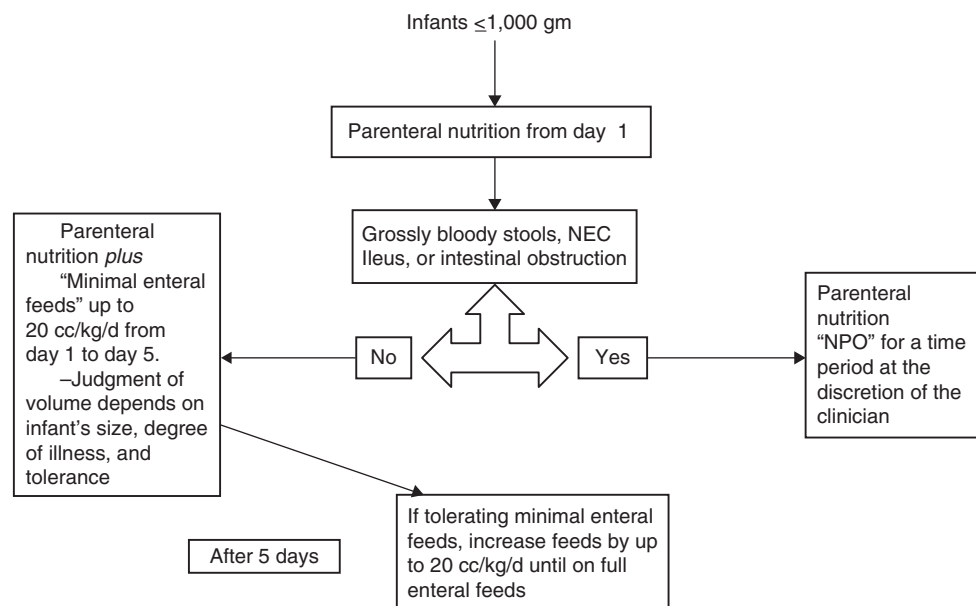


Figure 4 Suggested scheme for minimal enteral feedings in very low birth weight infants.

or absence of beneficial effects.⁹⁸ An example of such a group could be infants born to mothers with chorioamnionitis or other inflammatory conditions.

Omega-3 Fatty Acids

An important change to the composition of infant formula in the past several years has been the addition of LCPUFAs. The key LCPUFAs added have been DHA (22:6n-3) and arachidonic acid (AA, 20:4n-6). Both DHA and AA are found in human milk, and infants fed unsupplemented formulas have lower concentrations of DHA and AA in their plasma and erythrocytes than do infants who are breast-fed or fed supplemented formulas. It is also recognized that DHA and AA are integral structural components of all cells and may, therefore, have other health effects. Despite the voluminous information pertaining to potential benefits of the omega-3 LCPUFAs in both premature and term infants, the VLBW infant in the NICU receives only minimal quantities through the enteral route when receiving minimal enteral nutrition or parenteral route in the form of intravenous lipids, which are largely composed of the proinflammatory omega-6 fatty acids. Omega-3 LCPUFAs from fish oils and seed extracts decrease the production of inflammatory eicosanoids and cytokines. They act both directly, by replacing AA as an eicosanoid substrate and by inhibiting AA metabolism, and indirectly, by altering the expression of inflammatory genes through effects on transcription factor activation.⁹⁹ Thus omega-3 LCPUFAs are potentially useful anti-inflammatory agents and studies are needed to determine whether they would benefit patients, especially high-risk neonates.

Short-Chain Fatty Acids

SCFAs especially butyrate play central metabolic roles in maintaining the mucosal barrier in the gut. These SCFAs are a product of bacterial metabolism of carbohydrates, and may be responsible for the several of the beneficial effects of commensal bacteria or probiotics.⁴⁵ They can therefore be considered as “postbiotics.” There is currently major controversy pertaining to the use of probiotics for premature infants, partially based on safety concerns in providing live bacteria to immunocompromised hosts. Butyrate has the potential to offer some of the benefits of probiotic bacteria without the side effects. Butyrate has been shown to increase wound healing and to reduce inflammation in the small intestine. In the colon, butyrate is a dominant energy source for colonic epithelial cells and affects cellular proliferation and differentiation by yet unknown mechanisms.⁴⁵ Recent data suggest that the luminal provision of butyrate may be an appropriate means to improve wound healing in intestinal surgery and to ameliorate symptoms of inflammatory bowel diseases. Furthermore, butyrate has recently been found to stimulate intestinal L-cell enteroendocrine production of glucagon-like peptide-2, a highly

intestintrophic peptide.^{46,100} Very little research has been done on the effects of butyrate as a nutrient for the developing intestine.⁴⁵

SUMMARY

Several aspects of anatomic, physiologic, and biochemical development of the GI tract have been presented in this chapter. The importance of the GI tract beyond the classic paradigm of digestion and absorption of nutrients for optimization of growth should be evident. It should also be clear that the GI tract should be provided with luminal nutrients whenever possible. The developing GI tract has needs that cannot be met by merely nourishing the prematurely born infant similar to a term infant, or the way the fetus is nourished in utero. Optimization of nutrition for these infants based on the level of intestinal maturity continues to provide us with challenges with major implications for health during the lifetime of the individual.

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