Chapter 6

Breast Milk Decreases the Risk of Neonatal Necrotizing Enterocolitis

Ann M. Kosloske

1. Introduction

Necrotizing enterocolitis (NEC), a syndrome that strikes premature infants, has become a leading cause of infant mortality in the United States, Canada, and many other countries. The pathogenesis of NEC is poorly understood, although leading theories have implicated bacteria, intestinal ischemia, formula feedings, immaturity of the neonatal gut and combinations thereof (Santulli *et al.*, 1975, Lawrence *et al.*, 1982, Kosloske, 1990). This article summarizes our current understanding of NEC, including its history, clinical management, epidemiology and outcomes. Current theories of pathogenesis and prevention are outlined, with emphasis on the single agent with proven effectiveness for prevention: breast milk.

2. History of NEC

Case reports of "idiopathic" gastrointestinal perforation in newborn infants as early as 1825 (Siebold) probably included instances of NEC. Rossier *et al.* (1959) described a syndrome which they characterized as "l'enterocolite ulceronecrotique du premature." Fourteen of 15 premature infants in their series died with intestinal gangrene and perforation. In the 1960s and 1970s, NEC

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emerged as a major cause of infant mortality and morbidity, parallel to the establishment of neonatal intensive care units (NICU). Infants who formerly would have died of hyaline membrane disease, survived because of great improvements in neonatal care, only to develop potentially-fatal NEC. The mortality from NEC in the United States was calculated in 1988 at 1.3 cases per 1,000 live births, or 15–40% of cases (Holman *et al.*, 1989). It remains the most common gastrointestinal emergency of premature infants (Kliegman and Fanaroff, 1984) and one of the most serious of pediatric nosocomial (hospital-acquired) infections.

3. Clinical NEC

The clinical presentation consists of abdominal distention, gastrointestinal bleeding and pneumatosis intestinalis (air in the bowel wall) on abdominal X-ray. In addition, infants with severe NEC may have gas within the portal vein or pneumoperitoneum following intestinal perforation (Figure 1). The afflicted infant develops feeding intolerance, vomiting, and signs of sepsis. Typically, onset occurs near the end of the first week of life or during the second, although the syndrome may appear later, even in the second month. Ninety percent of infants are premature. When NEC is suspected, feedings are stopped and intensive medical therapy, including gastric suction, intravenous fluids and antibiotics, is begun. More than half of infants with NEC recover under medical management; the others, with more severe gut pathology, require emergency operation to prevent the development of intestinal gangrene or perforation (Kliegman and Fanaroff, 1984; Kosloske, 1997a,b).

The operative findings are those of ischemia of the bowel, varying from focal dusky areas to extensive gangrene (Figure 2). Affected loops may be pale gray from infarction or purple from hemorrhage into the gut wall. "Skip areas" of viable intestine may alternate with ischemic or frankly gangrenous segments. The ileocecal area is usually the most severely affected and the site of perforations. The process may involve ileum, colon, or jejunum, in that order, although rarely even stomach, duodenum, or rectum becomes necrotic (Kosloske, 1985). The surgical goal in NEC is resection of all the gangrenous bowel, with preservation of as much length as possible (Kosloske, 1997b; Albanese and Rowe, 1998). The majority of pediatric surgeons exteriorize the marginally viable ends of bowel as an enterostomy; primary anastomosis carries greater risk of complications (e.g., leak, stricture). The enterostomy is closed weeks or months later, after the infant has recovered from acute NEC.

The histologic findings of the resected bowel typically are those of ischemic necrosis, ranging from sloughing of the mucosa to full-thickness infarction (Figure 3). Acute inflammation may be minimal and confined to the margins of

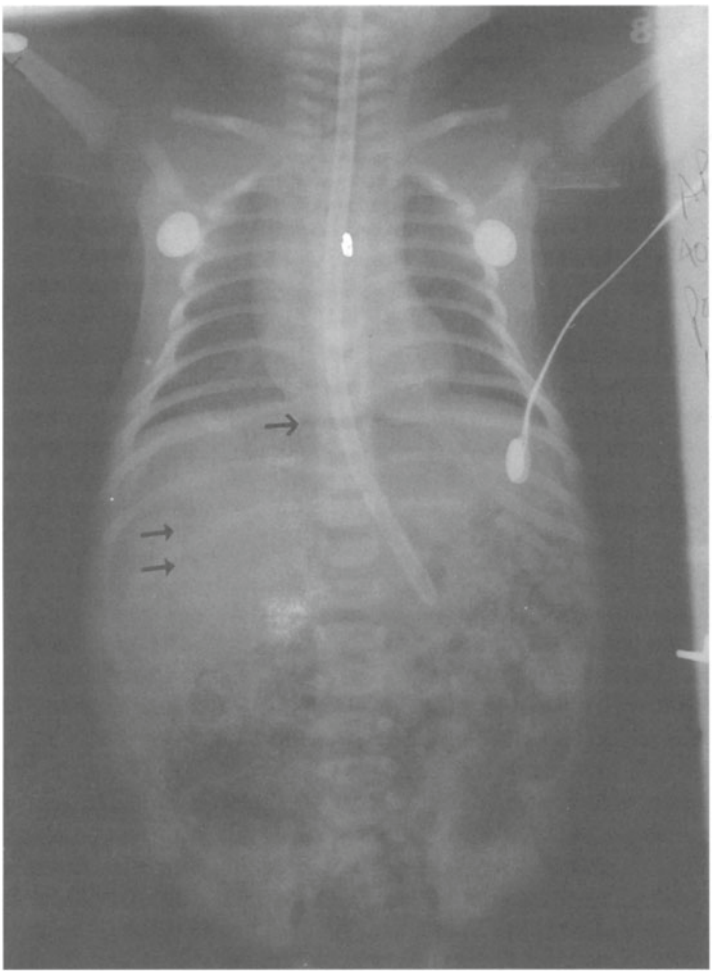

Figure 1. "Babygram" of an infant with NEC. Extensive pneumatosis intestinalis indicated by bubbly appearance of bowel throughout abdomen. Pneumoperitoneum (single arrow) and portal venous gas (double arrow) also are present.

the necrotic segment (Ballance *et al.*, 1990). Microthrombi are frequent, although major vessels are usually patent.

Improved survival from NEC over the past 3 decades has been attributed to earlier diagnosis and more effective supportive treatment of the premature infant. Currently, about two-thirds of surgical infants survive operation (Kosloske, 1997b; Albanese and Rowe, 1998), although some die later from complications

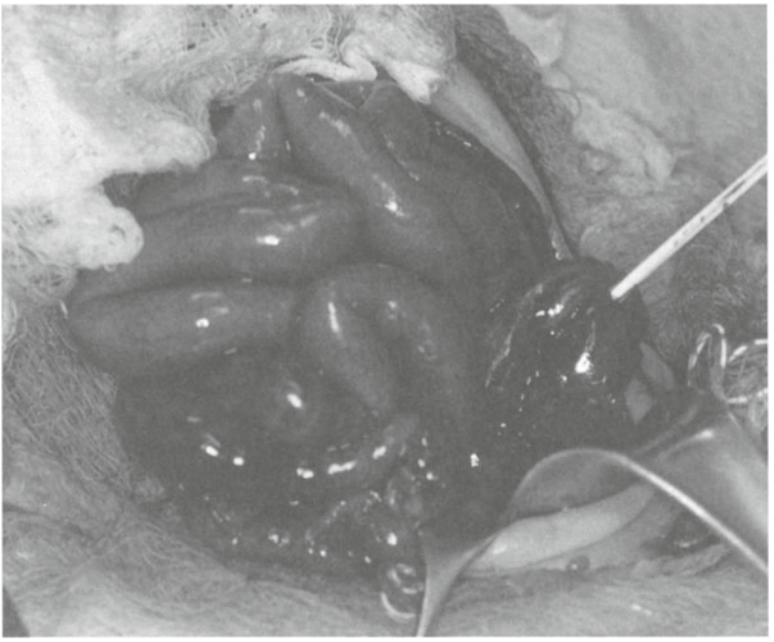

Figure 2. Photograph showing gangrenous loops of bowel in foreground and viable bowel in background. (Reprinted with permission, from: Kosloske, A.M. 1996. Necrotizing enterocolitis, in: *Newborn Surgery*, P. Puri, ed. Butterworth-Heinemann, Oxford, p.358.)

of NEC (e.g., short bowel syndrome, sepsis) or complications of prematurity (e.g., respiratory failure, sepsis). Since virtually all medically-treated infants survive the acute episode of NEC, the overall survival rate approximates 75–80%. The most common late complication of NEC is intestinal stricture, which develops in 15–25% of survivors (Kosloske *et al.*, 1980) from cicatricial healing of a site that sustained ischemic injury. Strictures usually require another operation for resection, although minor strictures distal to an enterostomy may be safely managed by balloon dilatation (Ball *et al.*, 1985).

4. Pathogenesis

Although the pathogenesis of NEC is poorly understood, a multifactorial process is invoked. The theories of Santulli *et al.* (1975) and of Lawrence *et al.* (1982) cite factors that may initiate NEC. Neither theory fully explains the clinical experience, although each has supportive clinical or experimental evidence.

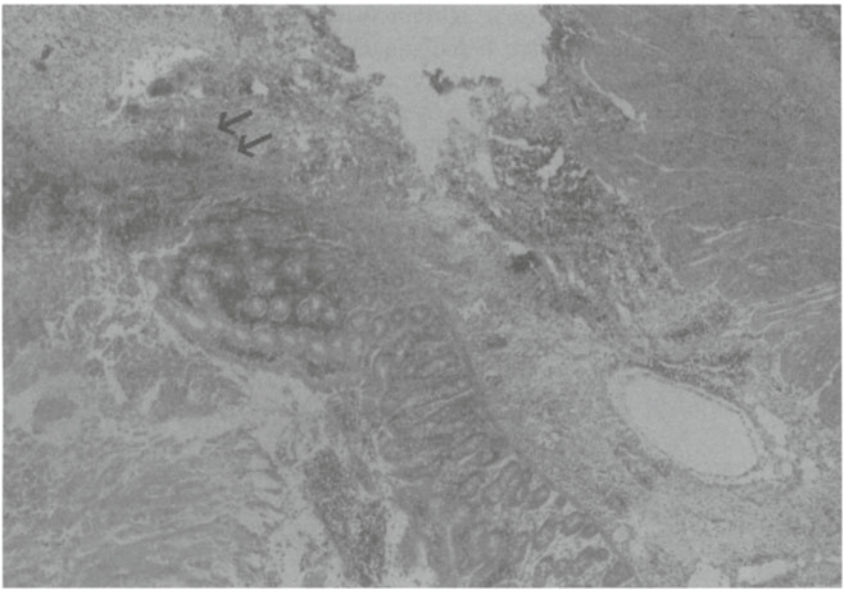

Figure 3. Photomicrograph of intestine resected for NEC, showing severe ischemic change of intestine, and focus of frank gangrene (arrows). (Hematoxylin & eosin; original magnification ×100.) (Reprinted with permission, from: Kosloske, A.M. 1997. Necrotizing enterocolitis, in: *Surgery of Infants and Children*, K.T. Oldham *et al.*, eds. Lippincott-Raven, Philadelphia, p.1202.)

Santulli *et al.* (1975) attributed NEC to the interaction of "three essential components": injury to the intestinal mucosa, the presence of bacteria, and the availability of a metabolic substrate, usually formula feedings, in the lumen of the gut. The hypothesis of Lawrence *et al.* (1982) emphasized the aberrant and delayed gut colonization of babies in the NICU, and the immunologic immaturity of the neonatal gut, which is vulnerable to direct damage from bacterial toxins in the gut lumen.

4.1. Ischemia

The intestinal injury which may precede NEC was linked by Lloyd (1969) with "the diving reflex," a physiologic defense mechanism that occurs in many species of birds and mammals in response to asphyxia (Scholander, 1963; Nowicki and Miller, 1992; Krissinger, 1994). Premature infants are prone to episodes of hypoxia associated with the immaturity of their lungs. During hypoxia, blood is shunted selectively to the heart and brain, away from the gut and other organs. After oxygenation is restored, reactive hyperemia occurs in

these organs. The resulting ischemia-reperfusion insult is believed to initiate NEC. Reperfusion of ischemic tissue triggers a chain of biochemical events leading to necrosis; a key step is the release of free radicals of oxygen, whose toxic chemical reactivity is a function of a single unpaired electron in the outer shell (Amoury, 1993). Thus, the enzyme xanthine oxidase, as well as hydrogen peroxide (H_2O_2) and cytotoxic free radicals, including superoxide (O_2^-) and the hydroxyl radical (HO^-), are released by polymorphonuclear neutrophils, and these inflammatory mediators generated during the process of injury may propagate the necrosis (Caplan and MacKendrick, 1994). Pharmacologic agents which are free radical anion scavengers (e.g., superoxide dismutase) or inhibitors of xanthine oxidase (e.g., allopurinol) might limit the necrosis induced by ischemia-reperfusion. Although several such agents have been studied experimentally, none has yet been evaluated in a clinical trial.

Other possible pathways for injury to the neonatal intestine include: (1) micro-emboli from umbilical arterial catheters inserted into the aorta for monitoring of arterial blood gases and blood pressure in the premature infant (Tyson *et al.*, 1976); (2) microthrombi from umbilical venous catheters which may communicate with the portal system; (3) decreased mesenteric flow associated with congenital heart defects (Leung *et al.*, 1988); (4) low-flow states from sepsis or other types of shock; (5) hyperviscosity; (6) a hyperosmolar flush of the mesenteric circulation from contrast media injected during cardiac catheterization (Cooke *et al.*, 1980); (7) hyperosmolar liquid in the bowel lumen (e.g., certain formulas or medications); (8) maternal use of cocaine, a potent vasoconstrictor. (Czyrko *et al.*, 1991); or (9) hypothermia, which experimentally produced intestinal ischemia more prolonged than that following hypoxia (Powell *et al.*, 1999). Episodes of hypoxic or other injury may be documented or inferred in the majority of preterm infants in NICU. It is not clear, however, why NEC develops in 5–8% of such infants, yet the remaining 92–95% of infants never develop the syndrome.

4.2. Bacteria

Although an infectious agent for NEC has been diligently sought, no single microbial pathogen has been found. The bacteria isolated from the blood or peritoneal fluid in NEC are, with few exceptions, members of the normal flora of the neonatal gut, most commonly *Klebsiella, E. coli*, or species of *Clostridia* (Guinan *et al.*, 1979; Kliegman *et al.*, 1979; Kosloske *et al.*, 1985; Speer *et al.*, 1976). *Clostridium perfringens*, the ultimate exotoxin-forming species, is associated with a fulminant, highly-lethal form of NEC (Kosloske *et al.*, 1978; Blakey *et al.*, 1985). The occurrence of NEC is usually sporadic, although occasionally it appears in epidemic-like clusters (Book *et al.*, 1977) during which a predomi-

nant organism may be isolated. Salmonella (Stein *et al.*, 1972), rotavirus (Rotbart *et al.*, 1988), and coronavirus (Chany *et al.*, 1982) are among the agents implicated in clusters of cases of NEC. The documentation of asymptomatic carriers in such epidemics of NEC suggests that impaired host resistance may be an important factor in the pathogenesis.

4.3. Feedings: Formula

Most infants with NEC had been fed a formula based on cow's milk. Although hyperosmolar formulae have been implicated in a few instances (de Lemos *et al.*, 1974, Book *et al.*, 1975), the mechanism by which isosmolar milk formulas may lead to NEC has not been established. A study by Engel and associates (1973) implicated feedings in the etiology of the pneumatosis intestinalis, i.e., the gas blebs which occur in the intestinal wall. Analysis of the gas, which was sampled from subserosal blebs at operation, showed that hydrogen predominated, a finding which supported the hypothesis that enteric bacteria, acting on formula as a substrate in the intestinal lumen, produce pneumatosis intestinalis. Conversely, pneumatosis intestinalis was found in only 57% of unfed infants who developed NEC (Marchildon *et al.*, 1982). Although some investigators have attributed NEC to overfeeding of premature infants, prospective clinical studies have failed to demonstrate that postponing enteral feeding prevents NEC. Rather, it may simply delay the onset of disease (Book *et al.*, 1976; Ostertag *et al.*, 1986). The option of total parenteral nutrition (TPN) may spare the gut but carries its own hazards (e.g., sepsis, cholestasis). The current trend in feeding the premature infant is by a combination of parenteral nutrition and low-volume enteral feeding (gut "priming"), which may induce adaptation of the immature gut. The role of feeding in the pathogenesis of NEC has been reviewed by Williams (1997).

4.4. Feedings: Breast Milk

NEC is rare in infants fed breast milk alone. In the animal model of Barlow *et al.* (1974), maternal milk protected neonatal rats from an intestinal necrosis that resembled NEC. Extrapolation of murine models to human infants is hazardous, however, because of immunological differences. In particular, rat pups receive their systemic immunoglobulin antibody (IgG) via the enteral route, whereas human infants receive IgG transplacentally. Human breast milk furnishes mucosal antibody (secretory IgA) to the neonatal intestine, as well as macrophages, lactoferrin, epidermal growth factor and other substances. The prospective multicenter study of Lucas and Cole (1990) documented a low incidence of NEC among premature babies fed breast milk alone. NEC was 6–10

times more common in those who received only formula feedings and three times more common in those who received formula plus breast milk. Pasteurized breast milk was as effective as raw milk, and the protective effect appeared to be strongest among infants 34–36 weeks gestation.

The precise mechanism by which human milk confers protection against NEC has yet to be elucidated. A variety of factors in milk have antimicrobial, anti-inflammatory or immunomodulating properties (Goldman, 1993; Buescher, 1994). In an experimental study (Go *et al.*, 1994), breast milk was compared to other feedings using bacterial translocation as a marker for the permeability of the mucosal barrier. Newborn rabbits fed breast milk had no bacterial translocation to the mesenteric lymph nodes or liver although 9% of the animals exhibited translocation to the spleen. In contrast, animals fed formula alone had incidences of 88%, 60% and 32% bacteria in the mesenteric nodes, liver and spleen, respectively. Stored breast milk was as effective as fresh breast milk in preventing bacterial translocation, suggesting that the cellular component of breast milk is not the protective element. Investigators from Michigan, using the same model, demonstrated a decrease in formula-associated bacterial translocation by the subcutaneous administration of epidermal growth factor (EGF) (Okuyama *et al.*, 1998), a component of breast milk, suggesting that EGF may be one of the protective elements. Improved barrier function was associated with an increase in the number of goblet cells in the mucosa of the intestinal villi.

4.5. Gut Colonization

Bacterial colonization of the neonatal gut begins by contact with the vaginal flora and is propagated by oral feedings and exposure to the environment. By 10 days of life normal infants are colonized by a range of aerobic and anaerobic species (Long and Swenson, 1977). In the aseptic environment of the NICU, however, colonization is delayed and limited to a small number of bacterial species. The use of antibiotics and sterile nursing practices in the NICU may exert the harmful effect of selection of an aberrant bacterial flora, characterized by antibiotic resistance and toxin production (Lawrence *et al.*, 1982). Damage to the mucosal enterocyte from such toxins may initiate NEC.

Bacterial overgrowth in the premature gut has been attributed to three different mechanisms: (1) formula feedings, (2) intestinal stasis, and (3) hypochlorhydria. Both clinical and experimental studies have demonstrated predominance of *Klebsiella* and other members of the family *Enterobacteriaceae* in the gut flora of formula-fed infants, in contrast to the *Lactobacilli* and *Bifidobacteria*, which predominate in the flora of breast-fed infants (Cooperstock and Zedd, 1983; Millar *et al.*, 1992).

Stasis in the neonatal gut may play a role in the pathogenesis. A surprisingly high incidence of postoperative NEC (18.5%) was reported in a series from

Michigan (Oldham *et al.*, 1988) following repair of gastroschisis, an abdominal wall defect often associated with delayed and sluggish gastrointestinal function. Jayanthi and associates (1998) from Great Ormond Street confirmed a protective role for breast milk in a series of 60 infants following repair of gastroschisis. Post-operative NEC developed in 30% of infants who were fed solely on formula, and in 5% of those who received formula plus breast milk. None of the 12 babies fed exclusively with breast milk developed postoperative NEC.

Premature infants normally have little gastric hydrochloric acid, and this may encourage bacterial overgrowth in the stomach. A clinical study by Carrion and Egan (1990) evaluated the effect of acidified feedings on the frequency of NEC. Infants who received acidified formula not only had decreased bacterial counts in the gastric juice, but also exhibited decreased occurrence of NEC. Only 1 of 34 premature infants who received acidified feedings developed NEC, whereas 8 of 34 control infants developed NEC. Experimental studies in germ-free models further support the role of bacteria in the pathogenesis of NEC. For example, Lawrence *et al.* (1982) produced necrotic enteritis by feeding any of six toxin-forming bacteria to germ-free rat pups. Moreover, Musemeche *et al.* (1986), using germ-free and gnotobiotic rats (i.e. animals colonized by a known flora), evaluated the relative importance of ischemia, bacteria and substrate in the pathogenesis of intestinal necrosis. In this important study, an isolated segment of ileum was subjected to ischemia by transient application of a microaneurysm clip. Formula, breast milk, or saline was injected into the lumen. A second, control segment without ischemia was created in each animal. Animals were sacrificed at 48 hrs and the severity of necrosis was graded by a pathologist. The most severe necrosis occurred in the gnotobiotic rats regardless of the duration of ischemia or the nature of the luminal contents. The germ-free rats showed little or no necrosis. Thus the most important of the three factors was the presence of bacteria.

4.6. The Immature Gut

The mucosal barrier to bacteria is incompletely developed in the newborn human infant, particularly in the premature infant. Udall (1990) cited evidence that immaturity of gastrointestinal host defense mechanisms is probably the single most important factor in predisposing infants to NEC. Intestinal B and T lymphocytes are decreased in number in newborn infants, compared with older infants (Udall, 1990). The terminal ileum at birth is permeable to the passage of intact macromolecules. Translocation of whole bacteria may occur with little impediment. Closure of the mucosal barrier coincides with the synthesis at the mucosal border of adequate levels of secretory IgA, and may not occur until 2 to 3 weeks of age. During this early vulnerable period, bacterial toxins generated in the bowel lumen may damage the enterocyte, thus initiating NEC (Lawrence *et*

al., 1982). A study by Bell *et al.* (1985) revealed higher levels of serum IgA in infants with NEC than in a control group of infants. This finding infers that there is a decrease in transport of IgA to the mucosal border in NEC, a conclusion consistent with the hypothesis of Lawrence *et al.* (1982).

The development of normal gastrointestinal motility depends upon the appearance of the interstitial cells of Cajal, a specialized type of cell in the gut wall distinct from the ganglion cell. The tyrosine-kinase receptor *c-kit* is a specific marker for the interstitial cells of Cajal. C-KIT$^+$ cells, such as mast cells and intraepithelial T-lymphocytes, are also important for immune system homeostasis. Investigators from Tokyo (Yamataka *et al.*, 1998) evaluated the role of *c-kit* in both experimental and clinical models. Strains of mice depleted of *c-kit* developed spontaneous intestinal mucosal erosions simulating NEC at 14 days of age. Ten infants with NEC showed a significant decrease in C-KIT$^+$ cells, compared to 10 controls. Six other infants with enteritis from conditions other than NEC had a significant increase of C-KIT$^+$ cells compared to controls.

Prospective clinical studies suggest that enhancement of gastrointestinal host defense may decrease the incidence of NEC. The British study which demonstrated the benefits of breast milk (Lucas and Cole, 1990) revealed a lower incidence of NEC in breastfed versus formula-fed prematures. Eibl *et al.* (1988) fed an immunoglobulin mixture (IgA and IgG) to infants at risk for NEC. They recorded a decreased incidence of NEC compared to control infants, although other investigators failed to confirm their results. Halac *et al.* (1990), in a prospective study in Argentina, evaluated the effect of corticosteroid therapy, which hastens maturation of developing tissues. They found a NEC incidence of 3.4% with prenatal steroid treatment and 6.9% with postnatal steroid treatment, compared to 14.4% in control infants. Substances that are trophic to the immature gut (e.g. epidermal growth factor, interleukin-11) or that function as nitric oxide donors (e.g. nitroglycerin), have shown promise in experimental models of bowel necrosis (Lawrence *et al.*, 1997; Du *et al.*, 1997; Graf *et al.*, 1997), but have yet to be tried clinically.

5. Epidemiology

Epidemiologic studies of risk factors for NEC have failed to identify any consistent cause, except prematurity (Stoll *et al.*, 1980; Ryder *et al.*, 1980). An interaction of risk factors is probable and quantitative aspects may be crucial. For example, severe intestinal ischemia might initiate injury in combination with highly pathogenic bacteria, an excess of potentially-damaging substrate or a very immature mucosal barrier. This unifying hypothesis (Kosloske, 1990) implies a threshhold of injury, which, if exceeded, results in NEC.

Although NEC occurs in developed countries throughout the world, its distribution is uneven (Kosloske, 1997a). For example, NEC is a problem in the United States, Canada, the United Kingdom, and Australia, but is rarely encountered in Switzerland, the Scandinavian countries and especially Japan, where a survey of NICUs showed an incidence that was 4 to 28 times lower than that reported for the United States (Shimura, 1990). In general, countries that enjoy low premature birth rates have few cases of NEC. Moreover, in developing countries in which NICU technology is not available, NEC is not a problem; however, older infants in developing countries may develop a type of necrotic enteritis resembling NEC following an episode of diarrhea and dehydration (Arseculeratne *et al.*, 1980). In some cases, the necrotic enteritis has been linked with overgrowth of a strain of *Clostridium perfringens* in the lumen of the bowel (Lawrence and Walker, 1976; Butler *et al.*, 1987).

6. Prevention

The prevention of NEC awaits a clearer understanding of its pathogenesis. There are few prospective, randomized studies of interventions which might prevent NEC. Randomization and concurrent controls are essential. Because NEC may occur in epidemics, historical controls may yield deceptive data, i.e., an intervention which was introduced as an epidemic subsided could be falsely credited with preventive efficacy (Kosloske, 1994). Prevention of the primary risk factor, prematurity, goes beyond medical issues, and would require societal and behavioral changes, e.g. universal prenatal care and cessation of maternal cigarette smoking and drug abuse. Clinical trials of interventions which might prevent NEC are hampered by its relatively low incidence (3–8% of NICU admissions), thus requiring a large sample size in order to achieve adequate statistical power. As many as 800 infants (400 treated, 400 controls), for example, may be required to achieve a statistical power of 0.8 (Hennekens and Buring, 1987). Thus far, only the British multicenter study of breast milk (Lucas and Cole, 1990), which entered 936 infants, meets such criteria. Their data support the recommendation of the American Academy of Pediatrics, which, for more than 50 years, has remained a staunch advocate of breast feeding as the optimal form of nutrition for infants (Gartner, 1997). Well-designed clinical trials are needed for evaluation of additional measures which might be beneficial to infants at risk for NEC.

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