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NECROTIZING ENTEROCOLITIS

The Search for a Unifying Pathogenic Theory Leading to Prevention

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HISTORICAL PERSPECTIVE AND SCOPE

In Schaffer's 1965 issue, *Diseases of the Newborn*,[103] no mention is made of necrotizing enterocolitis (NEC). In the 1971 issue,[104] it is discussed only briefly. Although references are made to an entity resembling NEC in the 19th and early 20th centuries,[43, 89, 95] it generally was not recognized as a disease that affects primarily premature infants until the 1950s and 1960s,[12, 80, 99, 107] which attests to its relatively infrequent incidence or recognition prior to modern neonatal intensive care.

During the past two decades, improvements in mechanical ventilatory support coupled with prevention and treatment for pulmonary immaturity have significantly improved the survival rate of low birth weight neonates. Concurrently, the incidence of NEC has increased in most centers and has emerged as the most common gastrointestinal emergency in neonates, affecting 2000 to 4000 newborns in the United States each year.[21, 22, 65, 102, 116, 125] Of these, 10% to 50% die, resulting in approximately 1000 infant deaths per year.[36] This number is close to the number of all US children fewer than 15 years of age who die of leukemia, or all children and adolescents fewer than 20 years who die of meningitis. The survivors of the acute episode of NEC frequently suffer with the effects of short bowel syndrome,[63, 64] which is a major cause of prolonged hospitalization and high medical expenses.

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Comprehensive information about the clinical aspects of NEC can be found in numerous textbooks and previously written reviews.* In this review, the clinical presentation and treatment of NEC is given only brief attention. The major focus is to provide an in-depth discussion of putative pathophysiologic events leading to NEC. An understanding of these events may hold the key for future prevention of this devastating disease.

CLINICAL PRESENTATION

Necrotizing enterocolitis is characterized by the following symptomatology and pathology: abdominal distention and tenderness, pneumatosis intestinalis, occult or frank blood in stools, intestinal gangrene, bowel perforation, sepsis, and shock. Based on clinical presentation, Bell and colleagues[11] originally described three levels of NEC, with stage 1 being suggestive, stage 2 being definitive, and stage 3 being severe (Table 1). Although stage 1 is nonspecific and may, in many instances, reflect feeding intolerance, sepsis, or gastrointestinal hemorrhage due to stress or other factors, stages 2 and 3 frequently are associated with considerable morbidity and mortality.

RADIOLOGIC FINDINGS

During the early stages of illness, the radiographic findings are nonspecific and include dilated bowel loops, generalized bowel distention, and bowel-wall thickening. A single persistent dilated loop should cause suspicion but is not a definitive sign of NEC or perforation.

Pneumatosis intestinalis, or gas in the bowel wall, in the appropriate clinical setting is usually diagnostic of NEC (Figs. 1 and 2). When pneumatosis intestinalis extends into the portal circulation (Fig. 3), it frequently is associated with severe disease. Pneumoperitoneum is frequently diagnostic of intestinal perforation with NEC. The air within the peritoneal cavity floats to the most nondependent area, which is just beneath the anterior upper abdominal wall in the supine patient. This air frequently outlines the falciform ligament (Fig. 4). Left lateral decubitus films can provide more information than supine films. Free air floats to the right surface of the peritoneal cavity and appears as a lucent area lateral to the liver edge. Careful serial reviews of left lateral decubitus films commonly are used to follow the progression of NEC and to determine whether perforation has occurred. Usually, pneumoperitoneum is considered an indication for surgery, whereas pneumatosis alone, without clinical deterioration of hematologic signs or acid-base status, usually is treated medically.

*References 11, 21, 22, 65, 84, 112, 116, and 125.

Table 1. MODIFIED BELL STAGING CRITERIA FOR NECROTIZING ENTEROCOLITIS

Stage	Classification	Systemic Signs	Intestinal Signs	Radiologic Signs
IA	Suspected NEC	Temperature instability, apnea, bradycardia, lethargy	Increased pregavage residuals, midabdominal distention, emesis, guaiac-positive stool	Normal or intestinal dilation, mild ileus
IB	Suspected NEC	Same as above	Bright red blood from rectum	Same as above
IIA	Proven NEC—mildly ill	Same as above	Same as above, plus absent bowel sounds, with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis
IIB	Proven NEC—moderately ill	Same as above, plus mild metabolic acidosis and mild thrombocytopenia	Same as above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as IIB, plus definite ascites
IIIA	Advanced NEC—severely ill, bowel intact	Same as IIB, plus hypotension bradycardia, severe apnea, combined respiratory and metabolic acidosis, disseminated intravascular coagulation, and neutropenia	Same as above, plus signs of generalized peritonitis, marked tenderness, and distention of abdomen	Same as IIB, plus definite ascites
IIIB	Advanced NEC—severely ill, bowel perforated	Same as IIIA	Same as IIIA	Same as IIB, plus pneumoperitoneum

NEC = necrotizing enterocolitis.

LABORATORY FEATURES

No individual laboratory features are diagnostic of NEC. Laboratory analysis primarily is used to confirm clinical diagnostic impressions and to judge progression of severity of disease.[108] Peripheral hematologic studies may reveal abnormally high or low white blood cell counts with a shift toward immature precursors. Progressively decreasing absolute

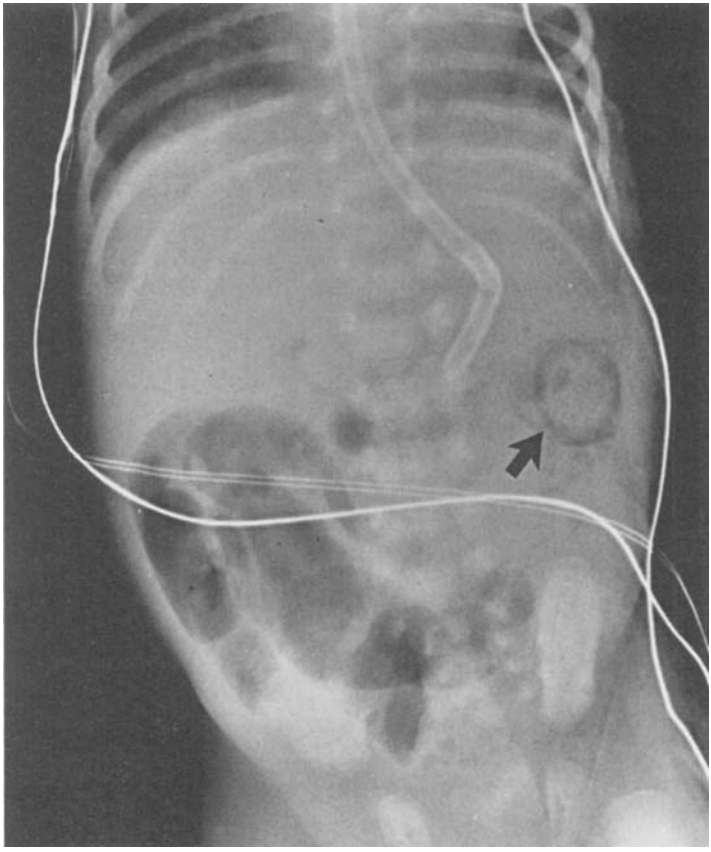

Figure 1. Several dilated air-filled loops of bowel in the right lower quadrant indicate a focal ileus. The arrow points to submucosal air in the splenic flexure.

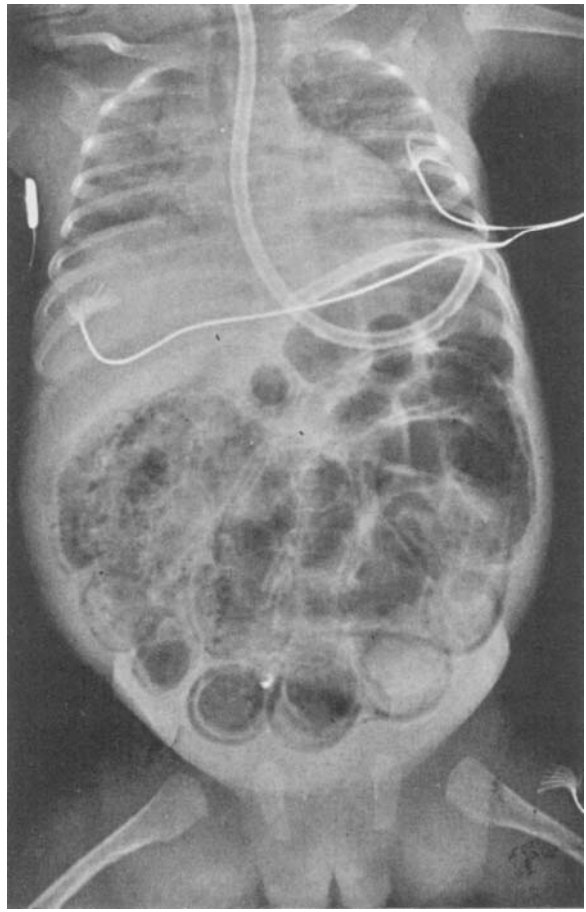

Figure 2. Film of the chest and abdomen shows massive air-distended bowel with diffuse intramural air.

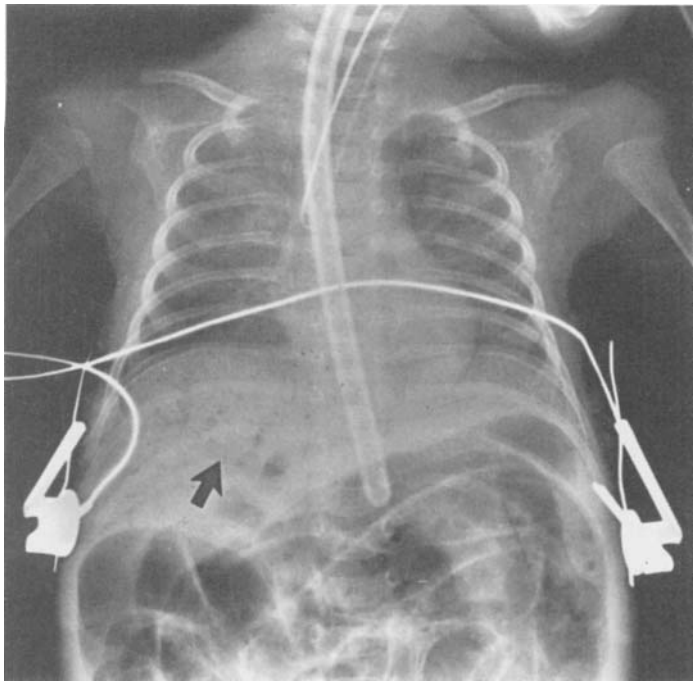

Figure 3. Air in the portal system *(arrow)*.

granulocyte count and thrombocytopenia suggest increasing severity of disease. If these are seen together with acidosis and severe electrolyte abnormalities, then the severity of NEC likely is progressing toward the necessity for surgical intervention.

PATHOLOGIC FINDINGS

The pathologic findings of NEC have been described by examination of the most severely affected patients who either died or who had intestinal perforation requiring resection of gangrenous bowel.[5, 65] Gross examination reveals involvement predominantly in the terminal ileum and proximal colon. In severe cases, however, the bowel from the stomach to the rectum may be involved. Histologic analysis reveals mucosal edema, hemorrhage, coagulation necrosis, and mucosal ulceration. The pathologic changes of NEC suggest a multifactorial cause, with bacterial overgrowth, inflammation, ischemia, and necrotic tissue all having a role.

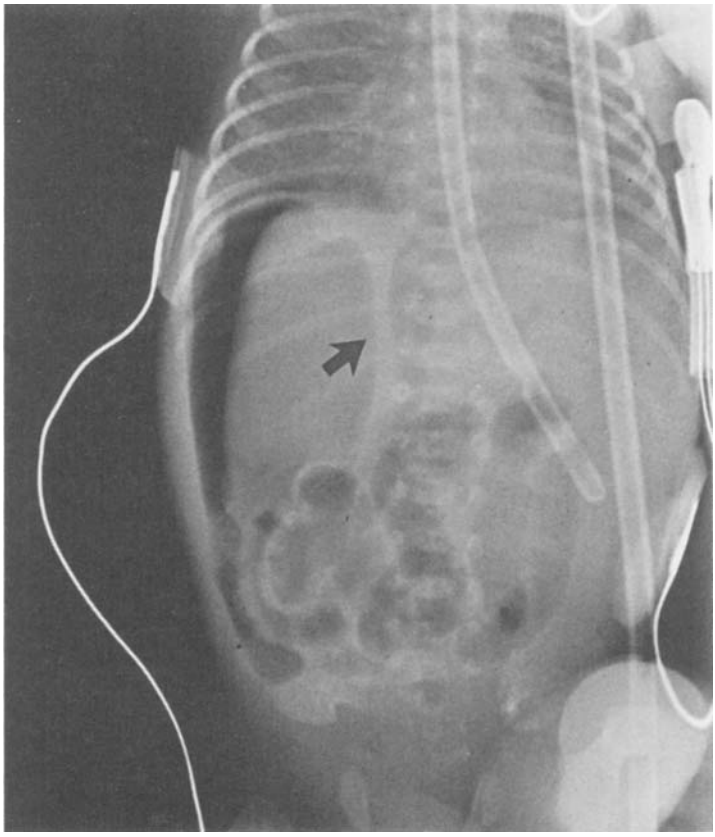

Figure 4. Left lateral decubitus film of the abdomen shows free intraperitoneal air from a bowel perforation. Free air is shown on both sides of the falciform ligament *(arrow)*.

CURRENT THERAPIES

Therapy for NEC is highly dependent on its severity. Bell's staging criteria[11] can be highly useful as guidelines. In stage 1, there is only a strong suspicion of the disease. Because of the potentially devastating progression of NEC, precautions need to be taken. In this scenario, clinical judgment based on the patient's condition should guide whether and how long the patient should be taken off enteral feedings, whether and how long intravenous antibiotics should be used, and how aggressively the patient is monitored with radiographs and laboratory tests. Once a definitive diagnosis is made but the patient has not progressed to surgical NEC (usually stage 3), the bowel should be decompressed using a large-bore orogastric tube with low intermittent suction. Careful attention needs to be placed on managing fluids and electrolytes because considerable third spacing of fluids with losses of sodium and protein

may occur. Potassium fluxes (high or low serum potassium) with acid-base imbalances are likely to occur. Renal failure is a frequent concomitant finding. Systemic antibiotic therapy, usually with ampicillin and gentamicin, are started after obtaining blood cultures. In cases in which resistant *Staphylococcus epidermidis* is suspected, vancomycin is used instead of ampicillin. When perforation is suspected, or has occurred, clindamycin or metronidazole is used to treat anaerobic infection. Intensive support of respiratory and circulatory status is provided, as are frequent monitoring of the abdominal status with left lateral decubitus radiographs and frequent monitoring of acid-base and hematologic status. A persistent acidosis with continued deterioration of platelet and white blood cell counts may be an indication for surgery even in the absence of overt perforation on radiography.

A review of surgical therapy of NEC is beyond the scope of this review. It should be remembered, however, that NEC is a disease that requires a carefully coordinated approach among neonatologists and pediatric surgeons. Because NEC can be such a rapidly progressive disease, the pediatric surgery section should be notified immediately whenever a diagnosis of NEC is made, even when only medical intervention is necessary at the time. This will allow for more rapid mobilization for surgery if and when it progresses to a surgical emergency.

RISK FACTORS AND PUTATIVE PATHOPHYSIOLOGY

The pathophysiology of NEC is not clearly understood. Although classically described as a disorder of sick premature infants in the neonatal intensive care unit (NICU), clinical experience shows that this disease occurs in several different settings and hosts. Although occasionally occurring in term infants and sick preterm infants who are not on enteral feeding and on ventilatory support, many cases of NEC occur in premature infants receiving intermediate or stepdown neonatal intensive care.[29, 112] Extremely premature infants (< 28 weeks' gestation) are at risk for the development of NEC for a protracted period. The risk is high until the infant has achieved a postconceptual age of 35 to 36 weeks and sometimes later depending on coincident gastrointestinal problems.[126, 127]

The current thinking about the pathophysiology of NEC is based on epidemiologic studies from which several important risk factors have been dissected. The putative risk factors that predominate are prematurity, aggressive enteral feedings, infectious agents, and hypoxic-ischemic insults.

PREMATURITY

The primary risk factor for NEC is prematurity because approximately 90% of cases occur in premature infants.[57, 65, 126] This disease rarely is seen in older children and adults. The cause of this age restriction

remains unclear, but an immature mucosal barrier and immune response, in addition to impaired circulatory dynamics, are thought to make premature neonates particularly susceptible.[62, 83] Immaturities of the developing gastrointestinal tract include immunologic factors, poor motility, reduced digestive absorptive function, increased membrane fluidity, low levels of protective mucus, and reduced regenerative capabilities, resulting in increased potential for tissue damage. Differences in gastrointestinal maturity that predispose premature infants to NEC include the following:

Immunologic factors
  Decreased IgA secretory component
  Decreased intestinal T lymphocytes
  Poor antibody response
Lumenal factors
  Lower H^+ ion output in stomach
  Low proteolytic enzyme activity
Immature intestinal barrier
  Mucin blanket composition
  Microvillus membrane biophysical properties and composition
  Higher permeability
Lower and less organized motility

Immunologic Factors

Necrotizing enterocolitis occurs most frequently in the terminal ileum and colon.[66] Large numbers of lymph follicles (Peyer's patches) are present in these regions.[88] An observation in rabbits of decreased IgA secretory component overlying the Peyer's patches of the ileum,[93] increased macromolecular uptake in this area[60, 87, 129] and translocation of bacteria in this region[13, 14, 56] are interesting in view of the frequent localization of NEC to this part of the intestine. Infant animals have also been shown to have decreased numbers of intestinal T lymphocytes,[50] indicating a compromise in cellular immunity early in life. The underdeveloped T-lymphocyte function may compromise immunologic surveillance and recognition of alterations in the membrane of an epithelial cell infected by a bacterial or viral agent. This compromises the ability to destroy the infected cell before the infectious agent can cross the basement membrane underlying the epithelium.[59]

Infants of less than 35 weeks' gestation have demonstrated a relatively poor response in the production of antibodies when compared with those of more than 35 weeks' gestation.[97] As the microbial agents or their toxins are allowed to enter the intestine, the premature newborn likely has a limited capability to respond immunologically.[73, 97]

Immature Lumenal Factors

Numerous immaturities in lumenal digestion exist in premature infants. One of the first lines of defense against ingested pathogens and

toxins is gastric acid.[119] Gastric-hydrogen ion output is low in the human neonate when compared with adults.[48, 83] This places these infants at an increased risk for colonization of enteric pathogens. Accordingly, acidification of feedings was demonstrated to decrease the incidence of NEC in one study.[27]

Proteolytic enzyme activity is also low. Enterokinase, the brush-border enzyme in the duodenum, converts inactive trypsinogen to active trypsin. The relatively low enterokinase activity[4, 132] and subsequently low tryptic activity might suppress the hydrolysis of various toxins that have the ability to damage the intestine. The occurrence of pigbel, a disease similar to NEC, seen in New Guinea, supports the importance of proteases as lumenal protective agents. Many highlanders of New Guinea subsist on a diet high in antiproteases. During festivals, massive quantities of uncooked meat are consumed after a prolonged fast. Enterocolitis develops, thought to be due to Clostridium beta toxin,[82] which passes into the intestine without the benefit of hydrolysis by endogenous proteases. The ensuing necrotic lesions in the gastrointestinal tract together with the propensity to perforate are similar to the intestinal pathology of NEC.

Immature Intestinal Epithelial Barrier

The intestinal mucin blanket also seems to be scant and have a different composition in newborn infants[2, 57, 102] when compared with adults, which makes the immature intestine more permeable to high molecular weight molecules.[39] This is also likely to facilitate bacterial adherence to the epithelium.[2, 57, 101]

The microvillus membrane composition and biophysical properties have been found to change with increasing maturation. Neonatal rabbits'[90] and rats'[51] microvillus membranes have higher lipid-protein ratios than those of adults. The fluidity (organization) of the microvillus membrane also changes as the animal matures and is shifted toward a more mature pattern by glucocorticoids administered to the mother antenatally or the infant postnatally.[85, 91]

Immature neonates have higher intestinal permeability than older children and adults. Preterm infants, especially those born at less than 33 weeks of gestation have higher serum concentrations of β-lactoglobulin than term neonates given equivalent milk feedings.[98] The permeability of the preterm human intestine to intact carbohydrate is greater in infants than in children or adults.[7] Using measurements of lactulose and rhamnose in the urine after ingesting milk containing these carbohydrates, some investigators have found that preterm neonates of 31 to 36 weeks' gestational age had an enhanced permeability of lactulose in the first week of life that changed to a more mature pattern in the second week. Infants born at a gestational age of 26 to 29 weeks had a more mature period of permeability at birth, followed by a temporary period of enhanced permeability at 3 to 4 weeks of age. Overall, there seems to

be a developmental pattern of decreased permeability with maturation. Breast-feeding is associated with a lower permeability to lactulose than formula-feeding,[120] which suggests that human milk contains factors that stimulate maturation of the small intestine. The specific nature of these factors remains speculative.

In addition to being relatively permeable to the uptake of macromolecules, the intestine of the newborn is also more permeable to the uptake of intact bacteria. When a breakdown in the mucosal barrier occurs, lumenal bacteria may translocate across the bowel wall into the blood or mesenteric lymph nodes.[47, 121] Translocation is likely to be responsible for many of the positive bacterial cultures obtained from the blood of neonates with NEC.[34]

Motility

The motility of the small intestine in premature infants is considerably lower and less organized than that in term infants.[121] This is caused by an intrinsic immaturity of the enteric nervous system that may cause delayed transit and subsequent bacterial overgrowth and distention. Although it is not clear specifically what role immature motility has in the pathogenesis of NEC, it likely contributes to the milieu in which the interaction of nutrients, immature host defenses, and other factors initiate the cascade of events culminating in NEC.[15]

AGGRESSIVE ENTERAL FEEDINGS

Necrotizing enterocolitis rarely, if ever, occurs in utero.[21, 65, 72] The fetus swallows up to 150 mL/kg/d of sterile amniotic fluid, which contains various nutrients, growth factors, and immunoglobulins in concentrations very different than those of formula or human milk.[94, 106] Although amniotic fluid provides a small amount of nutrients that can be utilized by the fetus, the majority of nutrition for the fetus comes from the placental circulation. In postnatal life, the previously sterile intestine becomes colonized with bacteria and is frequently stressed with relatively concentrated feedings. What accounts for the exclusive onset of NEC in the postnatal state? Perhaps, some of the components of amniotic fluid are highly protective to the fetal gastrointestinal (GI) tract. The fact that the GI tract of the fetus is not colonized with bacteria or exposed to a high volume of lumenal nutrients also likely has a role.

Although NEC occasionally occurs in infants who have never been fed,[3] it most frequently occurs in premature infants on enteral feedings and especially those whose enteral intakes are being aggressively increased. The advancement of formula feedings at rates greater than 20 kcal/kg/d has been found to be associated with an increase in the incidence of NEC.[3, 18, 20, 29, 118, 130] Despite the importance of aggressive enteral feedings in the pathogenesis of NEC, the finding in several

studies that "minimal enteral feeding" or priming the GI tract by a very slow intake using food as a trophic agent to stimulate GI mucosal development has not been associated with an increased incidence of NEC.[71, 76, 79, 111] Rather, there has been an improved tolerance to subsequent enteral feedings, lower incidence of cholestasis, and higher levels of potentially trophic gut hormones.

The possibility that human milk provides protection against NEC is supported by a multicenter trial designed to evaluate the effect of early diet on the incidence of NEC.[77] The study population, which was divided into infants fed only formula, those fed with formula plus expressed human milk, and those fed with expressed human milk alone, showed an incidence of 7.2%, 2.5%, and 1.2%, respectively. The theoretic basis of this is that human milk contains several known growth factors,[96] hormones,[67] macrophages, leukocytes, lymphocytes, immunoglobulins,[75] and enzymes.[110] Whether a similar protective effect would be incurred by banked donor human milk, which loses many potentially protective factors, remains speculative.

INFECTIOUS AGENTS

Prematurity and the presence of bacteria in the GI tract seem to be the most consistent risk factors associated with the development of NEC. Whether bacteria are primary inciting factors, merely permissive agents, or both in the pathogenic cascade leading to NEC remains unanswered.

Several lines of evidence support the thesis that infection is necessary for the development of NEC.[10, 19, 72, 100] This includes epidemiologic evidence for outbreaks suggestive of an infective process, the frequent isolation of infectious agents with NEC, and the decreased incidence of NEC resulting from preventive measures. Because many of the bacteria isolated from infants with NEC (from stool, blood, and peritoneal fluid) are organisms commonly found in the intestine, it is impossible to tell whether the presence of these bacteria is a causative factor or whether they are merely bystanders to a separate pathologic process. The latter is unlikely because of the large number of infants with NEC who exhibit overt pneumatosis intestinalis. If one uses Bell's criteria for the diagnosis of "proven" NEC (stage 2), the radiologic finding of pneumatosis is a necessary component. The gas in pneumatosis is thought to be derived primarily from bacterial fermentation.[43, 44, 61]

Kosloske[68, 69] presents bacteria as central factors in the "unifying hypothesis for pathogenesis and prevention of necrotizing enterocolitis." Bacteria commonly isolated from infants with NEC are gram-negative rods, including *Klebsiella* spp., *Escherichia coli, Enterobacter* spp., and *Pseudomonas* spp.[28] These bacteria can be isolated from blood, peritoneal fluid, intestinal tissues, and feces.[28, 81, 100, 122, 131] Other microorganisms associated with NEC are the bacteria *Clostridium difficile* and *Staphylococcus epidermidis* and the viruses coronavirus and rotavirus. The major arguments against etiologic roles for *C difficile* and *S epidermidis* in NEC

are the high rate of isolation of these bacteria or identification of their toxins in healthy, matched neonates and the lack of consistent recovery or detection of the bacteria or toxins in patients with NEC.[69]

Bacterial toxins also have been proposed as causes of NEC[105]; however, some potent toxins, such as *Clostridium* toxin, are isolated commonly from asymptomatic infants.[37] Because many different infectious agents have been associated with NEC, these agents may possess common virulence factors that may predispose susceptible hosts to the cascade of events involved in the pathophysiologic cascade of NEC. For example, many members of the family Enterobacteriaceae possess genes encoding Shiga-like toxin and cholera-like toxin.[1, 53, 115] Whether these genes are expressed by NEC pathogens and whether they have any role in disease are still unknown. Other possible common virulence factors may involve metabolic traits that predispose the host to other factors in the pathogenic cascade, such as a high capability to ferment lactose.[26]

A majority of very premature babies in the NICU are started on broad-spectrum IV antibiotic therapy shortly after birth during a "rule out sepsis" work-up. This can markedly alter the normal flora with which the neonate would become colonized.[9, 55] Rather than becoming colonized with *Lactobacillus* and other "normal" gut flora, resistant species indigenous to the NICU may colonize in the baby's intestine. Whether or how much of a role this has in the pathogenesis of NEC is not known. Recent studies also have shown that NEC-associated bacteria have a greater propensity than non-NEC–associated bacteria of the same species to prevent adherence of gram-positive bacteria to the GI tract and to cause disease in an animal model during coinfection with gram-positive isolates from the homologous child.[92] This could lead to an intestinal milieu consisting of a very large number of organisms more conducive to the development of NEC than would otherwise reside there. Other studies have shown that certain *Klebsiella* species that have a genetic predisposition (plasmid) for active fermentation of lactose also have the ability to cause NEC in isolated loops of rabbit small intestine.[26]

Infection-Associated Inflammatory Mediators

Similar to sepsis and adult respiratory distress syndrome, NEC seems to involve a final common pathway that includes the endogenous production of inflammatory mediators involved in the development of intestinal injury. Endotoxin lipopolysaccharide, platelet-activating factor (PAF), tumor necrosis factor (TNF), and other cytokines together with prostaglandins and leukotrienes are thought to be involved in the final common pathway of NEC pathogenesis.[25, 52]

Bacteria possess endotoxins that instigate the inflammatory cascade by activating PAF, TNF, and interleukin 1. PAF injected into the aorta of adult rats has been found to cause necrosis of the bowel[24] that can be prevented by pretreatment with PAF-acetylhydrolase[70] and can be exacerbated by a nitric oxide synthase inhibitor.[78] The generation of

these inflammatory mediators also has been shown to be decreased by the pretreatment with glucocorticoids,[24] which also have been shown to decrease the incidence of NEC if administered to the mother prior to the birth of her infant and in infants who are at high risk for the development of NEC.[6]

HYPOXIA-ISCHEMIA

Early theories of the pathogenesis of NEC suggested circulatory perturbations to be the most important determinants of pathogenesis.[74, 113] The physiologic support for linking ischemia to NEC relates a shunting of blood from the intestine during times of perinatal asphyxia. This was generated by the fact that many of the babies at that time who developed NEC also had antecedent episodes of perinatal distress.[54, 74, 113] Laboratory investigations in several animal models also demonstrated that a lack of perfusion to the bowel was an important antecedent to bowel necrosis.[30, 31, 117] This, coupled with the knowledge of a known redistribution of blood from the intestine to preserve blood flow to the brain, heart, and kidneys (in diving mammals, the "diving reflex"), led to the logical conclusion that hypoxia-ischemia is a major predisposing factor in the pathogenesis of NEC.[109] Other compelling evidence supporting ischemia as a major causative factor for NEC included histopathologic data that clearly indicated that ischemia occurs in the disease process.[5]

The clinical data that supported a hypoxic-ischemic role in the pathogenesis of NEC were largely anecdotal. The physiologic studies and clinical reports linking circulatory disturbances to the etiology of NEC recently have been disputed by both physiologic and clinical data.[86] Subsequent epidemiologic case-control studies showed that hypoxic-ischemic insults were not relevant risk factors in the development of NEC.[29, 33, 65, 114] The fact that so many cases of NEC occur in the intermediate or stepdown intensive care unit in babies who have never had known antecedent stresses, such as low Apgar scores, need for ventilatory support, umbilical catheters, polycythemia, or significant apneic episodes mitigates against hypoxia-ischemia as a major causative factor.

Even though both physiologic and clinical observations argue against a primary circulatory aberration as the cause of NEC, the histologic appearance of the disease, which includes coagulation necrosis suggesting ischemia as a component of the pathway, is compelling.[5] Hypoxia/ischemia to the bowel actually may be a secondary event that is mediated by other factors. It is tenable that inflammatory mediators released after endotoxin or bacterial invasion across the mucosa may lead to vasoconstriction and hypoxic-ischemic insults. The vascular control systems are known to be quite immature in these infants and could make the premature intestine more susceptible to the development of local tissue hypoxia.[86] Resting vascular resistance is known to be low in premature infants. The endothelium of the small intestine is responsible for the production of nitric oxide, a potent mediator known to maintain

low resting vascular resistance. If endothelial injury occurs, subsequent damage to the nitric oxide system likely could have a detrimental effect on local vascular tone, which could, at least theoretically, result in some of the tissue injury seen in NEC that is compatible with an ischemic insult.[78] Thus, other triggers, such as bacterial toxins or chemical irritation, could set off a cascade of events that could lead to endothelial disruption, altered nitric oxide production, and subsequently vascular compromise.[86] The specific relationship of endotoxin, PAF, and nitric oxide together with other mediators remains to be elucidated. Whether elucidation of this pathway might result in a potential treatment or prevention for NEC is discussed later.

PREVENTION

Several measures are commonly used to prevent NEC outbreaks. The first includes careful epidemic precautions when an outbreak of NEC is suspected. These precautions are predicated on an increased awareness of NEC being present in the NICU in more than one infant and a suspicion that microbial agents are involved in the pathogenesis of the outbreak. Preventive measures include strict infection-control measures to prevent fecal and oral spread; cohorting of patients, contacts, and personnel; and using a decreased threshold for early intervention, such as placing babies nulla per os (NPO), providing antibiotics while cultures are pending, and delaying or slowing enteral feedings while an outbreak is suspected. Although oral antimicrobial agents have been used for prophylaxis in contacts to interrupt the outbreak,[8, 40, 49] the possibility of emergence of resistant organisms limits their routine long-term use.[41]

Although glucocorticoids are not routinely used for the prophylaxis of NEC as they are being used for the prevention of respiratory distress syndrome (RDS), the data are highly suggestive of their potential benefit.[6, 32, 51, 58] The mechanism for this is unclear but may involve a maturational effect on the microvillus membrane,[85, 91] an increase in the activities of several enzymes involved in digestion and absorption of nutrients,[83] or a decrease in mucosal inflammation. In reality, many of the babies at highest risk for NEC are also at highest risk for RDS and thus may actually be receiving prophylaxis antenatally with glucocorticoids via the mother. The decreased incidence of NEC in babies of mothers who are treated with antenatal steroids[6, 32] should provide an additional indication for the administration of corticosteroids to mothers in preterm labor. Whether the routine use of postnatal glucocorticoid prophylaxis for NEC would be effective without causing other risks to the infant, such as increasing the incidence of sepsis or catabolism, is not known, and further studies are needed to determine the risks versus the benefits of such prophylaxis.

Providing human milk to premature infants has been shown to decrease the incidence of NEC in one large multicenter study.[77] Fresh human milk is composed of numerous immunoprotective factors, such as immunoglobulins, lysozyme, lactoferrin, macrophages, lymphocytes,

and neutrophils.[23] The possibility of banked or refrigerated donor milk providing benefit is less clear because the cellular components and immunoglobulins are compromised by the pasteurization or freezing process. Another potentially beneficial component of human milk recently discovered is PAF acetyl hydrolase (PAF-AH–102). This enzyme inhibits the activity of PAF, which is likely to be a significant mediator in the pathophysiologic cascade of NEC. Because of the likely protective effect against NEC and other infections in addition to the nutritional and psychosocial benefits afforded by human milk, it is the author's opinion that premature infants should be provided with his or her own mother's fresh milk whenever feasible.

The finding that an IgG-IgA preparation obtained from human serum provided protection against NEC in premature infants in a randomized trial is compelling.[42] Whether this preparation acts to directly prevent bacterial invasion into the gut or to counteract the release of cytokines from monocytes[128] is not known. This study should be repeated and confirmed at a larger scale, preferably in a multicenter format prior to recommending routine use of this modality for the prevention of NEC.

As previously discussed, the aggressive institution of enteral feedings in preterm infants is a frequent risk factor associated with NEC. This has, in many cases, caused neonatologists to institute an overly cautious approach to enteral feedings whereby the baby is placed NPO for several weeks after birth and nourished only by the parenteral route. This approach is known to cause atrophy of the intestinal mucosa and may be highly detrimental in that the onset of NEC may be delayed only after feedings are finally instituted. An alternative approach with several different names, including "minimal enteral nutrition," "intestinal priming," and "hypocaloric feedings," has been studied in a controlled randomized manner[38, 79] involving use of enteral nutrition to provide topical nutrition to the small intestine during the first week or two of life while providing most of the nutritional needs for the infant by the parenteral route. It is usually reserved for infants weighing less than 1500 grams. "Priming" is not contraindicated while the infant is on ventilatory support or using umbilical catheters. The enteral feedings are increased so that the infant is on full enteral intake in the third to fourth week of life. This approach has been found to improve tolerance to subsequent enteral intake, stimulate early secretion of several intestinal hormones that may be trophic in the intestinal mucosa, and improve motility.[16] Despite demonstrating these advantages, the numbers from the individual studies were too small to determine whether this approach caused a significant decrease in the incidence of NEC.

The osmolarity, carbohydrate composition, and pH of the enteral intake also have been implicated in the pathogenesis of NEC. Formulas with high osmolarity have been shown to increase the incidence of NEC in human infants[17] and cause mucosal injury in animal models.[35] Many oral medications, such as vitamin preparations, use hyperosmolar vehicles[123, 124] that potentially could cause osmotic injury to the bowel. The

carbohydrate found in human milk and most commercial infant formulas is lactose. There is some controversy as to whether the premature small intestine has the capability to hydrolyze lactose to the same degree as that of the term infant.[83] Also, the lactose not hydrolyzed by the intestine may be hydrolyzed by bacteria, with the subsequent fermentative generation of hydrogen gas and short-chain fatty acids.[61] The presence of certain bacteria that have an especially efficient capability to ferment lactose using beta-galactosidase could lead to a high concentration of lumenal short-chain fatty acids, which could alter the pH of the small bowel and cause mucosal breakdown, with the invasion of bacteria into the mucosa and production of pneumatosis intestinalis.[26] In the upper GI tract, the situation is different than in the distal. Many premature infants are hypochlorhydric and do not respond as quickly as older children or adults to a meal with brisk production of gastric acid and peptic proteases.[48, 83] This acid environment is an effective barrier to many potentially pathogenic microorganisms. A prospective double-blind study that compared the incidence of NEC in a group of infants fed a regular formula to infants fed a formula supplemented with acid to achieve a pH between 3 and 4 showed a lower incidence of NEC in the acid-supplemented group.[27] These studies should be confirmed before considering routine use of this method.

Another method that has been attempted as prophylaxis against NEC included the provision of oral aminoglycosides (kanamycin or gentamicin). Several of the studies suggested a significant decrease in the incidence of NEC in the groups given oral antibiotics[8, 40, 49] compared with controls. The long-term use of these antibiotics unfortunately was associated with the emergence of resistant strains of *Staphylococcus, Klebsiella,* and *E coli.*[41] These findings have discouraged the routine use of long-term oral antibiotic therapy for the prophylaxis of NEC.

Table 2 shows the pathophysiologic features together with the corresponding preventative measures that have been attempted or are being used in the prevention of NEC.

FUTURE APPROACHES FOR PREVENTION

As the pathophysiologic cascade for NEC becomes more defined and the tools for investigative science improve, the likelihood of finding

Table 2. PREVENTION BASED ON PATHOPHYSIOLOGY

Pathophysiologic Feature	Preventive Measures
Microbial infection	Oral antibiotics Formula acidification Epidemiologic control Human milk IgG-IgA
Immature intestine	Glucocorticoids Human milk Intestinal priming

effective prophylaxis for NEC increases. The potential for prophylaxis of NEC can be found at several levels. It is reasonable to assume that interventions aimed at the more proximal events of the cascade offer a greater likelihood for effective prophylaxis. Areas for possible intervention include the following:

Identification of a common microbial agent or common bacterial virulence factors
Maturation of the intestine using trophic agents
Interference in the host-response to the inciting factors

At this time, a single microbial agent known to cause NEC has not been identified. Some as yet unidentified microbes may be involved. Viruses such as coronavirus and rotavirus have been implicated in the pathogenesis of NEC but have not been consistently found. As previously discussed, it is highly likely that bacteria are involved, not necessarily as triggering agents, but as agents that enable the pathophysiologic cascade of NEC to proceed. The fact that so many premature neonates are placed on broad-spectrum antibiotics shortly after birth to rule out sepsis changes the gut flora toward resistant organisms that are likely to possess virulence factors very different than those in the normal flora. Perhaps strong consideration should not be given to pre-emptively treating all premature babies with antibiotics for 3 days pending culture results as is so commonly done. Obviously, the risk of sepsis needs to be carefully weighed against the likelihood of altering the bacterial microenvironment in each individual baby. Some bacteria cultured from babies with NEC have been found to have the capability to inhibit adherence of normal gut flora to CaCo-2 cells in culture.[92] Other studies have shown that certain bacteria isolated from neonates with NEC have a highly efficient capability to ferment lactose in the presence of hydrolytic products of casein.[26] These bacteria in turn were found to have the capability to produce severe intestinal damage in isolated rabbit intestinal loops. When isolated from the original bacterium and cloned into an otherwise nonpathogenic bacterium, the previously nonpathogenic bacterium becomes a rapid lactose fermenter and is able to cause disease in the isolated rabbit intestinal loops. There are likely to be numerous other virulence traits possessed by NEC-associated bacteria that have not yet been identified. Identification of these traits, proof of their pathogenicity, and subsequent production of neither active nor passive immunity against them offer the likelihood of specific prophylaxis against NEC.

The finding that human milk is likely to be protective against NEC has stimulated the search for various immunologic and nonimmunologic components of human milk that could be responsible. A specific nutrient, such as glutamine or nucleotides, may be involved. Glutamine is one of the most important metabolic substrates for the GI tract. This amino acid has been found to attenuate various forms of enterocolitis in animal models and to decrease permeability to lactulose and mannitol in adults. It is thought to become an essential amino acid during times

of stress. It is not normally supplied to these babies in appreciable quantities by either the enteral or parenteral route because most premature neonates are not provided with appreciable enteral nutrition, and neonatal TPN solutions do not contain glutamine. Other growth factors and hormones that are known to directly improve GI function and maturity, such as epidermal growth factor, insulin-like growth factor 1, and thyroid hormone, also offer a fertile area for future investigation for the prevention of NEC.

Understanding the specific steps in the inflammatory cascade resulting in NEC also should offer opportunities for further intervention. Inhibitors of PAF, stimulators or inhibitors of nitric oxide synthase, cytokines, prostaglandins, and leukotrienes may have a role in the future prevention of NEC. Although intervening in the inflammatory cascade seems to offer hope, the disease may be so far advanced that rescue at this stage is no longer likely, and prevention may be impossible. Intervention into more proximal events in the pathologic cascade by altering the lumenal milieu by immunizing against microbial virulence factors or maturing the intestinal barrier should offer the most promise for prophylaxis against NEC.

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