# CHAPTER 94

# Necrotizing Enterocolitis

Karl G. Sylvester, Gigi Y. Liu, and Craig T. Albanese

Necrotizing enterocolitis (NEC) is an acquired inflammatory disease that affects the gut of newborn infants nearly exclusively. Despite decades of research, NEC remains a leading cause of infant morbidity and mortality in neonatal intensive care units (NICUs). Increased rates of preterm birth and advances in neonatal care have contributed to a growing population of infants at risk for NEC. It is now the most common newborn surgical emergency and is associated with significant morbidity and mortality that exceeds all other gastrointestinal (GI) conditions requiring surgical intervention. Although the precise pathogenesis remains incompletely understood, clinical progress in recent years portends a shift in focus to prevention and the earlier identification of those infants most at risk or with progressive disease. Despite the recent completion of two successful prospective trials, the optimum surgical management of advanced disease with perforation remains controversial.

## Historical Perspective

Dating to at least 1888, there are several reports describing pathologic findings of intestinal perforation in neonates as the cause of death suggestive of NEC.[1–3] The first report of a successfully treated infant with a localized ileal perforation that was described as a disease resembling NEC is attributed to Agerty[4] in 1943. Subsequently, in 1953 Schmid and Quaiser[5] first used the term *necrotizing enterocolitis*. In 1964 Berdon[6] reported the clinical and radiographic findings of 21 patients with NEC. Then in 1975 Santulli[7] first hypothesized that the development of NEC had three essential components: injury to the intestinal mucosa, the presence of bacteria, and the availability of a metabolic substrate (to be taken as the presence of enteral feedings). This characterization remains a central tenet of our understanding of the overall pathophysiology of NEC (discussed later). Over the past several decades the management of infants with NEC has evolved from aggressive early operation to supportive care with the increasing realization that most infants can be managed, at least initially, nonoperatively.

The subsequent seminal work of Bell and colleagues[8] codified a severity-based classification scheme that is widely accepted due to its simplicity and clinical utility in suggesting therapy on the basis of likely outcomes. Bell's criteria (Table 94-1) can be summarized as indicating clinical findings suspicious for NEC (Bell's stage I), definitive NEC (Bell's stage II), and advanced NEC (Bell's stage III). In general, a stage I infant manifests clinical criteria that raise suspicion without definitive evidence such as pneumatosis intestinalis or bloody stool. In stage II, or definitive disease, there is nearly always evidence for pneumatosis intestinalis (Fig. 94-1). The hallmark of advanced disease is the appearance of pneumoperitoneum or other clinical findings to suggest irreversible tissue damage with perforation. In 1979 the International Classification of Diseases established a code for death from NEC, thereby allowing more precise epidemiologic and outcome analyses. Currently, the optimum surgical approach in order to realize the best short- and long-term outcomes in infants with intestinal perforation secondary to NEC remains the subject of intense scrutiny via ongoing clinical trials.

## Incidence

Although the overall reported incidence of NEC among newborn infants is relatively low and reported to fall between 5% and 10%.[9–11] The true incidence is unknown given a number of either early or suspicious cases of NEC that cannot be accurately tabulated. Conversely, the number of infants that are under consideration for either having or developing NEC can be quite high and is directly related to degree of prematurity. Perhaps more puzzling, the incidence of NEC varies significantly within the United States and throughout the developed world. For example, the worldwide incidence of NEC in very-low-birth-weight (VLBW, <1500 g) infants varies from 1% to 2% in Japan, 7% in Austria, 10% in Greece, 14% in Argentina, and 28% in Hong Kong.[12–16] The reasons for these disparities are unclear but are likely multifactorial including biologic (e.g., genetic) and environmental (e.g., variation in practice patterns). Irrespective of geographic reporting location, it is clear the incidence of NEC varies according to degree of prematurity and birth weight. NEC accounts for 1% to 7% of all NICU admissions in the United States, or 1 to 3 cases per 1000 live births.[9,17,18] In VLBW infants, the disease occurs in approximately 10% to 12%, but ranges between 2% and 22%, depending on the center of inquiry.[11,15,19]

**TABLE 94-1**

**Modified Bell Staging Criteria for Necrotizing Enterocolitis**

| Stage | Systemic Signs | Abdominal Signs | Radiographic Signs |
|---|---|---|---|
| IA<br>Suspected | Temperature instability, apnea, bradycardia, lethargy | Gastric retention, abdominal distention, emesis, heme-positive stool | Normal or intestinal dilation, mild ileus |
| IB<br>Suspected | Same as above | Grossly bloody stool | Same as above |
| IIA<br>Definite, mildly ill | Same as above | Same as above, plus absent bowel sounds with or without abdominal tenderness | Intestinal dilation, ileus, pneumatosis intestinalis |
| IIB<br>Definite, moderately ill | Same as above, plus mild metabolic acidosis and thrombocytopenia | Same as above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass | Same as IIA, plus ascites |
| IIIA<br>Advanced, severely ill, intact bowel | Same as IIB, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, DIC, and neutropenia | Same as above, plus signs of peritonitis, marked tenderness, and abdominal distention | Same as IIA, plus ascites |
| IIIB<br>Advanced, severely ill, perforated bowel | Same as IIIA | Same as IIIA | Same as above, plus pneumoperitoneum |

Modified from Neu J: Necrotizing enterocolitis: The search for a unifying pathogenic theory leading to prevention. Pediatr Clin North Am 1996;43:409-432 and Caplan MS, Jilling T: New concepts in necrotizing enterocolitis. Curr Opin Pediatr 2001;13:111-115.
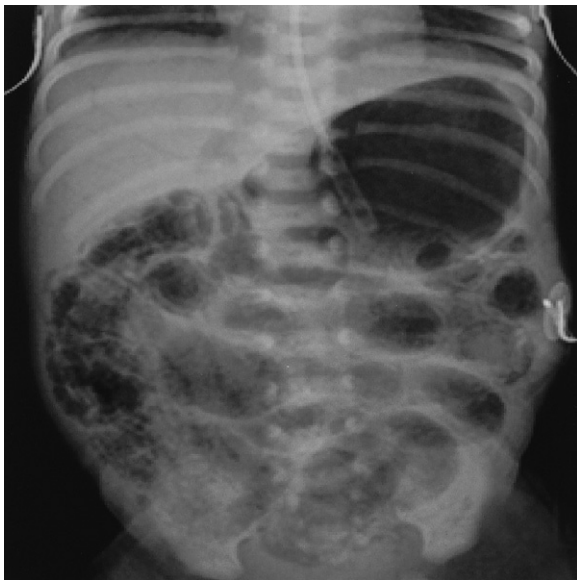


**FIGURE 94-1** Plain abdominal radiograph demonstrating extensive pneumatosis intestinalis (cystic and linear) and an arborizing pattern of air over the liver shadow representing gas dispersed within the radicles of the portal venous system.

Several confounding factors may account for the variability in incidence reporting including the number and survival of low-birth-weight (LBW) infants, the source of patient referrals (inborn or outborn), and the diagnostic criteria used to establish definitive NEC. A relatively large multicenter survey of VLBW infants noted an incidence of 10.1% for definite NEC (stage II) and 17.2% for suspected NEC (stage I), although there was considerable intercenter variability.[20] In another study, the incidence of definite NEC versus suspected NEC was 8.6% and 18.6%, respectively.[18] The observed variability in these and many other series may be underestimating the true frequency of NEC because the incidence is often defined as the total number of cases of NEC divided by the total number of patients admitted to the NICU. Thus these figures may include many premature infants who die within the first several days of life and before enteric feedings and therefore are unlikely attributable to NEC. In consideration of this, a notable historical study that excluded early neonatal deaths and included only infants who had been fed reported an incidence of 15%.[21]

# Epidemiology and Pathogenesis

## EPIDEMIOLOGY

### Age and Maturity

NEC is predominantly a disease of premature LBW infants rather than those who are small for gestational age. It is estimated that only 7% to 13% of all NEC cases occur in full-term infants.[22,23] Kliegman and Fanaroff[24] reported that the mean gestational age of 123 patients with NEC was 31 weeks (average birth weight, 1460 g). Infants with extremely low birth weight (ELBW) (<1000 g) and those 28 weeks' gestational age or younger are at greatest risk.[25,26] In a large multicenter prospective study from the NICHD Neonatal Research Network involving 4438 infants weighing between 501 and 1500 g, Lemons[11] demonstrated an inverse relationship between the incidence of NEC and birth weight. Specifically, the incidence of NEC was highest in infants weighing between 501 and 750 g (14%) and declined with increasing weight: 751 to 1000 g (9%), 1001 to 1250 g (5%), and 1251 to 1500 g (3%). These findings have been confirmed by others[23,27,28] and extended to document an inverse relationship between the age at onset of NEC and gestational age. Infants in whom NEC developed in the first week of life were more mature (average gestational age, 36.1 weeks) than those in whom NEC developed after

1 week of age (average gestational age, 33.4 weeks). Complications were more common, and the mortality rate was higher in patients with early-onset disease. Wilson[29] calculated the birth weight–specific, weekly attack rate in patients with NEC and found the risk period for NEC decreased as birth weight increased. They found a consistent pattern of sharply declining risk with attainment of age equivalent to 35 to 36 weeks' gestation. From these observations the authors speculated that functional maturation of the GI tract may play a principal role in determining the risk for NEC.

## Feedings

Approximately 90% of NEC cases develop in infants after feedings are initiated.[30–32] In a longitudinal cohort study reviewing the incidence of NEC for a 3-year period before and after implementing a "standardized feeding schedule" for infants weighing between 1250 and 2500 g and less than 35 weeks' gestation, Kamitsuka[33] reported an 84% reduction in the risk for NEC. Other studies have also suggested an association between an increase in the incidence of NEC and advancement of formula feedings at rates greater than 20 kcal/kg/day.[34,35] Despite these reports, randomized trials failed to demonstrate any difference in the incidence of NEC related to fast versus slow, early versus delayed, or continuous versus intermittent bolus feedings.[36–39] In a randomized trial involving 185 VLBW infants in which slow (15-mL/kg/day increments; 10-day schedule to full feeding) and fast (35-mL/kg/day increments; 5-day schedule to full feeding) feeding advancements were compared, Rayyis[39] demonstrated no significant difference in the incidence of NEC (13% vs. 9%), perforation (4% vs. 2%), and mortality (2% vs. 3%) between groups. In a review of randomized trials comparing continuous versus intermittent bolus tube feeding for premature infants weighing less than 1500 g, Premji and Chessell[38] found no significant differences in the incidence of NEC between the two groups.

In a randomized trial investigating the incidence of NEC in VLBW infants assigned to receive either minimal-volume feeding (20 mL/kg/day) for 10 days before advancing to full-volume feeding or a standard feeding advancement protocol (starting at 20 mL/kg and increasing by 20 mL/kg/day to full-volume feeding), Berseth[40] reported a significantly lower incidence of NEC in the minimal-volume group than in the standard group (1.4% vs. 10%). The prolonged use of "trophic feeding" volumes is thought to trigger maturation of GI structure and function. This study reinforces previous studies that gut stimulation protocols are beneficial to VLBW infants[41] and that initiation of a minimal-volume feeding protocol for 7 to 10 days followed by modest advancement of feeding may greatly reduce the incidence of NEC.[42] However, there are no contemporary studies that specifically address this issue.

## Pharmacologic Agents

Indomethacin is commonly used in premature infants to treat a hemodynamically significant patent ductus arteriosus (PDA). Indomethacin blocks prostaglandin synthetase, thus causing vasoconstriction. Spontaneous gastrointestinal perforation (SIP) and NEC have been noted in LBW infants treated with high-dose indomethacin.[43,44] It has been postulated that indomethacin increases mesenteric vascular resistance and reduces mesenteric blood flow by 16% to 20%.[45] Norton[46] demonstrated that the use of indomethacin as a tocolytic agent

was associated with an increased incidence of NEC in babies delivered before 30 weeks' gestation (mean age at delivery, 27.6 weeks), although indomethacin did not increase the incidence of NEC in babies born after 32 weeks' gestation. Two randomized controlled trials involving more than 500 LBW premature infants receiving early low-dose indomethacin versus placebo for closure of a PDA demonstrated no difference in the subsequent incidence of NEC.[47,48] According to several recent Cochrane Reviews, ibuprofen appears to be as effective as indomethacin in leading to a PDA closure and with fewer reported cases of NEC or SIP.[49] Interestingly, although the continuous infusion of indomethacin for a PDA leads to fewer alterations in cerebral, renal, and mesenteric blood flow compared with bolus infusion, to date, insufficient evidence exists to demonstrate that this results in a lowered risk of NEC or SIP.[50] Earlier indomethacin may be associated with increased incidence of SIP but protection from NEC. Moreover, a PDA itself may predispose to NEC, independent of indomethacin.

## Cytokines and Growth Factors

Cytokines and growth factors play a critical role in mediating the interaction among enterocytes, endothelial cells, fibroblasts, and inflammatory cells that together are critical to the overall cellular pathophysiology of NEC. These soluble factors direct cellular proliferation, maturation, chemotaxis, and activation in both the local gastrointestinal milieu and systemically to effect the onset and progression of NEC (Table 94-2).[51–56]

### Growth Factors

Epidermal growth factor (EGF) is known to be an important trophic factor for the developing GI tract and has been shown to be present in high concentration in human breast milk.[57,58] Typically, EGF is secreted into the gut lumen primarily by the salivary and Brunner glands of the duodenum and binds to EGF receptors that have been demonstrated throughout the fetal and neonatal intestine, especially on the basolateral membrane of enterocytes.[59–64] EGF enhances proliferation and differentiation of epithelial cells but also has significant effects on healing of damaged mucosa and on intestinal adaptation after injury.[62,65–67] Significantly reduced levels of salivary and serum EGF have been demonstrated in premature infants in whom NEC developed versus age-matched controls.[68,69] Furthermore, lower levels of salivary EGF during the first week of life were associated with an increased incidence of NEC in a recent clinical trial[70] involving 327 premature and term neonates. Similarly, inactivation of the EGF receptor in knockout mice has been shown to result in hemorrhagic enteritis that is histologically similar to NEC.[71]

Several animal studies that administered EGF provide insight to the molecular mechanism underlying EGF-mediated protection against NEC. In studies using a neonatal rat model of NEC that involved asphyxia and cold stress, enterally administered supplements of EGF have been shown to significantly decrease the incidence and severity of NEC in rat pups[72] through down-regulation of the proinflammatory interleukin-18 (IL-18) and increased production of the anti-inflammatory cytokine IL-10.[73] Supplement of EGF in two rat models has successfully reduced intestinal epithelial cell apoptosis in the ileum, decreased intestinal permeability,

**TABLE 94-2**

**Summary of Important Growth Factors and Cytokines Contributing to the Pathogenesis of NEC**

| Cytokines | Functions | Proinflammatory | Antiinflammatory | Protective Effects in Guts | Trophic Effects in Enterocytes |
|---|---|---|---|---|---|
| EGF and HB-EGF | • Proliferation and differentiation of epithelial cells<br>• Healing of damaged mucosa | No | Yes | Yes | Yes |
| Epo | • RBC proliferation | No | No | Yes | Yes |
| IL-1β | • Macrophage activation, neutrophil recruitment, expression of endothelium adhesion molecules<br>• Production of IL-6, IL-8, PGE2 | Yes | No | Unknown | Yes |
| IL-4 | • T- and B-cell and macrophage regulation<br>• Differentiation of CD4 T cell into Th2 cells | Yes | Yes | Yes | Unknown |
| IL-6 | • Production of acute phase proteins, B-cell growth, T-cell proliferation, metalloproteinases, and GM-CSF | Yes | Unknown | Unknown | Unknown |
| IL-8 | • Attraction of neutrophils and basophils to site of inflammation | Yes | No | No | Unknown |
| IL-10 | • Decreases macrophage activation<br>• Inhibition of proinflammatory cytokine production | No | Yes | Yes | Unknown |
| IL-11 | • Increases megakaryocyte and macrophage production | No | Yes | Yes | Yes |
| IL-12 | • Production of IFN-γ, Th1 and NK cell proliferation<br>• Cytotoxic T lymphocyte and Th1 cell differentiation<br>• Macrophage activation and production of complement-fixing antibodies<br>• Up-regulation of IL-18 receptor | Yes | Unknown | Unknown | Unknown |
| IL-18 | • IFN-γ and B-cell antibody production<br>• Enhanced NK cell cytotoxic activity<br>• Activation and migration of neutrophils, phagocytosis, and integrin expression | Yes | No | Unknown | Unknown |
| NO | • Regulation of leukocyte-endothelial interaction and platelet aggregation and adhesion<br>• Apoptosis from peroxynitrite when NO reacts with superoxide | Yes | Yes | Yes | No |
| TNF-α | • Cytokine release of IL-1β, IL-6, IL-8, NO, PGE2, matrix metalloproteases, PAF, and TXA2<br>• Inhibition of the release of glucocorticoids, TGF-β, and IL-10<br>• Apoptosis induction<br>• Neutrophil activation and recruitment | Yes | Unknown | Unknown | Unknown |
| PAF | • Mesenteric vasoconstriction<br>• Capillary leakage and increased intestinal mucosal permeability<br>• Neutrophils and platelet activation | Yes | Unknown | Unknown | Unknown |
| COX2 | • Synthesis of proinflammatory prostaglandins | Yes | Unknown | Unknown | Unknown |

Modified from Ledbetter DJ, Juul SE: Necrotizing enterocolitis and hematopoietic cytokines. Clin Perinatol 2000;27:697.

increased mucin production by goblet cells, and improved overall intestinal structure.[74,75]

Heparin-binding epidermal-like growth factor (HB-EGF) is a member of the EGF family of growth factors. HB-EGF initially was identified in conditioned medium of macrophage-like cells as a mitogen for fibroblasts and smooth muscle cells.[76] The presence of HB-EGF has been reported in both human amniotic fluid and milk.[77,78] An HB-EGF knockout mouse model has shown significantly increased intestinal permeability, delayed onset of angiogenesis, and increased incidence and severity of NEC.[79,80] According to several animal studies,[78,81–84] HB-EGF has been demonstrated to protect developing intestinal epithelium from hypoxic necrosis and cytokine-induced apoptosis, as well as to exert its cytoprotective effects via decreased reactive oxygen and nitrogen species production. Interestingly, simultaneous administration of both EGF and HB-EGF did not result in any additional protective effect against NEC.[83]

Erythropoietin (Epo) is a peptide produced by the kidneys that regulates red blood cell production in response to anemia. Since development of the recombinant protein, rEpo has become widely used in the NICU.[85,86] Epo has been found in human breast milk, and functional Epo receptors have been demonstrated in fetal and neonatal small intestine, thus

suggesting a possible role in GI development.[87–89] In a retrospective study comparing 260 VLBW infants who received recombinant Epo (rEpo) with 233 matched controls, Ledbetter and Juul[90] demonstrated a significantly lower incidence of NEC in the rEpo group (4.6%) than in the control group (10.8%). Studies in neonatal rats given rEpo enterally have demonstrated a dose-dependent increase in intestinal mucosa villus surface area and increased cellular proliferation, thus suggesting a role of rEpo as a trophic factor in the developing small intestine.[91] In a neonatal rat model of NEC involving exposure to hypoxia and reoxygenation, pretreatment with intraperitoneal injections of rEpo resulted in significantly decreased mucosal inflammation and necrosis, which was suggested to be mediated by decreased production of nitric oxide (NO).[92]

## Cytokines

Cytokines are endogenous mediators of the inflammatory cascade. Proinflammatory and antiinflammatory cytokine production is tightly regulated by complex feedback mechanisms to maintain homeostasis.[93,94] Overproduction of either may have significant untoward effects. Overproduction of proinflammatory cytokines (IL-1, IL-2, IL-6, IL-8, IL-12, tumor necrosis factor-alpha [TNF-$\alpha$], interferon) and platelet-activating factor (PAF) may lead to shock, multiorgan failure, and death.[95,96] Overproduction of antiinflammatory cytokines (IL-4, IL-10, IL-11) may result in excessive suppression of immune function.[97,98] A number of different inflammatory mediators have been implicated in the pathogenesis of NEC, several of which are highlighted later.[99,100]

IL-1$\beta$ in the gut can be found in macrophages, neutrophils, intestinal epithelial cells, endothelial cells, fibroblasts, dendritic cells, smooth muscle cells, and enteric glia.[101,102] Microbial products, inflammation, and TNF-$\alpha$ trigger its release.[101,102] Upon binding to the IL-1 receptor, IL-1$\beta$ and its receptor activate the transcription factor NF-$\kappa$B, which triggers release of acute phase proteins, IL-6, IL-8, and PGE$_2$.[101,102] Elevated IL-1$\beta$ has been detected in full-thickness specimens of NEC intestine.[103] Edelson[104] detected a greater level of IL-1 receptors late in the course of severe NEC. Both IL-1$\beta$ and IL-1 receptor may serve as markers for progressive disease.

Levels of IL-6 increase in the presence of microbes, microbial products, TNF-$\alpha$, and IL-1$\beta$.[101,105] Upon binding to IL-6 receptors, which are only expressed on hepatocytes and some leukocytes, IL-6 triggers the STAT-4 pathway, resulting in production of acute phase proteins, B cell growth, antibody production, T-cell proliferation, and enhanced activity of hematopoietic growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF).[101,105] Furthermore, it leads to the production of tissue inhibitors of metalloproteinases and inhibition of superoxide production.[106] Elevated IL-6 has been reported in the plasma and stool of babies with NEC,[107] but its mRNA expression in surgical intestinal specimens is not any higher than the controls.[105]

IL-8 is released in response to LPS, TNF-$\alpha$, and IL-1$\beta$.[108] After binding to the chemokine receptors CXCR1 and CXCR2, which signal through phospholipase C and PI3-kinase, respectively, IL-8 is responsible for neutrophil activation and migration into tissues, as well as the production of acute phase proteins.[108] In a study evaluating serial serum levels of two proinflammatory cytokines (IL-1$\beta$, IL-8) and two antiinflammatory cytokines (IL-1 receptor antagonist [IL-ra], IL-10) in infants with NEC, Edelson and colleagues[104] demonstrated significantly higher levels of IL-8 and IL-10 in infants with severe NEC from the onset of disease through 24 hours than in infants with less severe NEC. Nadler[109] investigated the pattern of cytokine expression in infants undergoing resection for severe NEC and infants undergoing intestinal resection for other inflammatory conditions. Significant up-regulation of IL-8 mRNA was seen in the specimens from infants with NEC as compared with controls.

IL-12 is released in response to bacteria, bacterial products, and viruses and exerts its effect on T cells and NK cells upon binding to its receptor.[110] Its immunologic functions include the following: IFN-$\gamma$ production, Th1 and NK cell proliferation, cytotoxic T lymphocyte and Th1 cell differentiation, macrophage activation, and production of complement-fixing antibodies.[110] Halpern[111] localized IL-12 via immunohistochemistry to monocytes in the intestinal mucosa and lamina propria in a neonatal rat model of NEC.

Tissue IL-18 levels peak in response to lipopolysaccharide (LPS or endotoxin), Fas ligand, gram-positive bacteria exotoxins, and IL-12 and subsequently induce the production of TNF-$\alpha$ and IL-1$\beta$.[112] Binding to the IL-18 receptor results in NF-$\kappa$B activation and promotes Th1 or Th2 lineage maturation depending on the underlying genetic influence and cytokine environment.[113] Heninger[114] discovered infants with stage III NEC or above to have a higher frequency of the IL-18607 AA genotype, thus suggesting that this variation may be used for predicting the outcome of NEC.

IL-4, produced by Th2 cells, mast cells, B cells, and stroma cells, is a key regulator in humoral and adaptive immunity.[100,120] It has been demonstrated to possess cytoprotective effects in human intestinal epithelial cells by reducing bacterial translocation, increasing leukocyte superoxide production, and inducing decay-accelerating factor, which protects the host from the attack of autologous complement activation.[115,116] Genetic studies by Treszl[117] revealed that infants with NEC were less likely to possess the IL-4 receptor $\alpha$-chain mutant allele compared with infants without NEC. Treszl[117] speculated that this mutant allele is associated with enhanced transduction of IL-4 signals, which shifts the development of lymphocytes to a more pronounced Th2 state.

IL-10, produced by Th2 cells, monocytes, and B cells, is an inhibitor of proinflammatory cytokine production and of several accessory cell functions of the macrophage, T cell, and natural killer (NK) cell lines.[118] Furthermore, IL-10 has also been shown to decrease the production of metalloproteinases[119] and suppress iNOS mRNA and NO expression in small bowel, liver, and serum.[120] It is postulated that diminished production of IL-10 in preterm infants resulted in persistent up-regulation of the inflammatory response and therefore increased susceptibility in the preterm neonate to long-term tissue damage after acute inflammatory conditions.[121] Although Edelson and colleagues[104] found significantly higher levels of IL-10 in infants with severe NEC from the onset of disease through 24 hours than in infants with less severe NEC, in a neonatal rat hypoxia-reoxygenation model of NEC, recombinant IL-10 administered subcutaneously was found to significantly attenuate the extent of intestinal injury when compared with control animals, thus suggesting a protective effect of its antiinflammatory properties.[122] Taken together, these findings indicate that IL-10 has the potential to be a marker of severe disease and may

be considered a therapeutic target to function as a strong cytokine inhibitor. The high level of IL-10 in severe NEC, in contradistinction to less progressive NEC, suggests a significant role for antiinflammatory mediators in the pathophysiology of NEC to dampen the inflammatory response.

IL-11 or adipogenesis inhibitory factor is a member of the IL-6 type cytokine family that uses the gp130 receptor subunit for intracellular signal transduction. IL-11 has been shown to be a pleiotropic cytokine that promotes epithelial regeneration and enhances adaptation after bowel resection.[123] Subcutaneous administration of recombinant IL-11 in rats undergoing placement of a defunctionalized (Thiry-Vella) loop of intestine or massive small bowel resection has resulted in prevention of mucosal atrophy in the defunctionalized loop and enhanced mucosal adaptation and absorptive function in the remaining intestine after resection.[124,125] In addition, Nadler[109] noted significant up-regulation of IL-11 mRNA in infants undergoing resection for severe NEC and found an inverse correlation between IL-11 expression and the likelihood of pan-necrosis, thus suggesting that IL-11 secretion may be an adaptive response to limit the extent of intestinal damage.

TNF-α has many proinflammatory effects including neutrophil activation, induction of leukocyte and endothelial adhesion molecules, and induction of other cytokines such as PAF.[126] TNF-α has been demonstrated to produce profound hypotension and severe intestinal necrosis similar to NEC in animals. This effect has been shown to be mediated by PAF and attenuated by PAF receptor antagonists.[127] Confirmatory studies in human neonates with NEC identified significantly elevated plasma levels of TNF-α compared with controls.[99] Pentoxifylline, a drug that has multiple effects including inhibition of production of TNF-α, was shown to reduce bowel necrosis in an adult rat ischemia-reperfusion model of bowel injury, as well as the incidence of NEC in a neonatal rat model.[128] Furthermore, two separate studies[129,130] have demonstrated a significant reduction in the severity of NEC in neonatal rats after intraperitoneal TNF-α antibody prophylaxis. However, the A(-308) and A(-238) allele variants of the promoter region of the TNF-α gene, which were reportedly associated with higher TNF-α production, fail to show any influence on the risk and course of NEC in VLBW infants.[131]

A tremendous amount of investigation has been performed to define the role of nitric oxide (NO) in the pathogenesis of a number of different inflammatory processes. Evidence supports a possible dichotomous function of NO as both a beneficial and a detrimental molecule, especially with regard to the GI tract.[132,133] NO is produced from arginine by three isoforms of nitric oxide synthase (NOS). NOS-1 (neuronal, nNOS) and NOS-3 (endothelial, eNOS) are constitutively present at low levels in the small intestine. NOS-2 (inducible, iNOS), which is expressed in the myenteric plexus, endothelial cells, gastric epithelial cells, and enterocytes,[134,135] is a form that can be induced in response to inflammatory cytokines.[136,137] The constitutive forms of NOS and constitutive levels of NO have been demonstrated to modulate a number of important functions in the GI tract including maintenance of mucosal integrity, regulation of mucosal permeability, modulation of water and electrolyte transport, regulation of blood flow, regulation of motility, and inhibition of leukocyte adhesion and activation.[138–142]

Inhibition of NO synthesis in a variety of animal models of intestinal injury induced by ischemia-reperfusion, LPS, or PAF has resulted in marked exacerbation of mucosal injury.[143–148] Administration of exogenous sources of NO including L-arginine, sodium nitroprusside, and nitroglycerin greatly attenuates these detrimental effects.[149–152] In a prospective study of 53 premature infants, Zamora[153] demonstrated a significantly lower plasma arginine level at the time of diagnosis in infants in whom NEC developed compared with controls. In the only randomized, prospective, placebo-controlled study of VLBW infants assigned to receive either daily L-arginine supplements (261 mg/kg) or placebo for the first 28 days of life, Amin[154,155] found a significantly lower incidence of NEC in the supplement group (6.7%) than in the control group (27.3%). Throughout the study, the group that received arginine supplementation had significantly higher mean plasma arginine levels than the control group did. Interestingly, in both groups the infants in whom NEC developed had significantly lower plasma arginine levels at the time of diagnosis than their respective peers did. It is not known whether the decreased arginine levels represent increased utilization for NO production, consumption for the synthesis of other proteins, or decreased enteral absorption. This study suggests the possible benefits of arginine supplementation and potentially other NO donors in the prevention of NEC.

The inducible form of NO synthase (NOS-2, iNOS) is induced in response to inflammatory cytokines. Within several hours of stimulation, NOS-2 expression and activity within the intestinal epithelium increase up to 15-fold and result in the production of large amounts of NO.[135] Although the low levels of NO produced by the constitutive isoforms of NOS may play a homeostatic role in the GI tract, sustained release of NO as a result of up-regulation of NOS-2 has been suggested to have deleterious effects by inducing cellular injury and failure of the mucosal barrier.[135,156,157] This is thought to occur by the reaction of excess NO with superoxide ($O_2-$) to produce peroxynitrite ($ONOO-$), a potent reactive nitrogen intermediate that may trigger cytotoxic processes including lipid peroxidation and DNA damage.[157–159] In a prospective study investigating NOS-2 expression in the intestine of infants undergoing resection for NEC, Ford[160] demonstrated marked up-regulation of NOS-2 gene expression in the intestinal epithelium and increased apoptosis of enterocytes in the apical villi. In addition, increased levels of nitrotyrosine residues were detected in the apical villi, thus suggesting that the mucosal injury and increased apoptosis were mediated through the formation of NO and peroxynitrite. In a neonatal rat hypoxia model of NEC in which breast milk–fed animals were compared with formula-fed animals, Nadler and colleagues[161] demonstrated a significantly higher incidence of NEC, NOS-2 expression, and enterocyte apoptosis along with decreased IL-12 mRNA in the formula-fed group than in the breast milk–fed group. The decreased IL-12 expression is thought to be mediated by NO and theorized to contribute to intestinal injury by attenuating bacterial clearance. Furthermore, Whithouse has shown in a rat model that the progression of NEC to intestinal ischemia is associated with a shift from nitric oxide to superoxide production by the intestinal vascular endothelium.[162]

PAF, an endogenous phospholipid inflammatory mediator, has been shown to play an important role in the pathophysiology of intestinal injury in both animal and human studies.[12] PAF has diverse biologic effects including

mesenteric vasoconstriction, capillary leakage, increased intestinal mucosal permeability, and neutrophil and platelet activation.[163–165] Clinically, increased PAF levels have been demonstrated in formula-fed premature infants, as well as in those in whom NEC developed.[100,166] In a neonatal rat model of NEC, Caplan[167] showed that intestinal PAF concentrations, intestinal phospholipase A2 (PAF-synthesizing enzyme) mRNA expression, and intestinal PAF receptor mRNA expression are all elevated. In other animal experiments, exogenous PAF administration results in severe bowel necrosis[168]; endogenous intestinal production of PAF is up-regulated in response to various stimuli including LPS, hypoxia, and TNF-α[127,169–171]; and administration of PAF receptor antagonist, or PAF acetylhydrolase (PAF-degrading enzyme), reduces the risk for NEC.[170,172,173] In human studies, PAF acetylhydrolase activity has been demonstrated to be present in human breast milk; it has also been shown to be decreased in neonates, with levels approaching adult enzyme activity at around 6 weeks of life, and has been found to be deficient in infants with NEC.[99,174,175] In a prospective study of 164 infants at risk for NEC, Rabinowitz[176] monitored serial plasma levels of PAF and PAF-related lipids (PAF-LL) to investigate the changes that occur with NEC. There was a significantly higher peak in PAF-LL levels in infants in whom NEC developed than in controls. In addition, rising PAF-LL levels were positively correlated with progression of the severity of NEC; these levels returned to baseline levels during recovery. With the studies mentioned, a systematic review[56] of serologic tests in diagnosing NEC suggested that PAF is one of the better performing markers for NEC with a sensitivity of 92% and specificity of 84% despite the considerable heterogeneity between the studies.

Cyclooxygenase (COX) catalyzes the rate-limiting step of arachidonic acid metabolism into prostaglandins, leukotrienes, and thromboxanes.[177] Two isoforms of the COX enzyme have been identified. COX-1 is constitutively expressed in many tissues including the GI tract.[178] COX-2 is the inducible form that is expressed in inflammatory conditions of the GI tract such as inflammatory bowel disease.[179,180] Proinflammatory cytokines (IL-1, IL-6, TNF-α), as well as the proinflammatory transcription NF-κB, have been shown to increase COX-2 expression.[181] NF-κB is an important protein in the activation of a number of inflammatory mediators and cytokines.[182] A marked increase in COX-2 expression has been demonstrated in intestine resected from infants with severe NEC.[183] To elucidate the mechanisms involved in COX-2 expression in NEC,[183] Chung demonstrated a coordinated induction of NF-κB activation and COX-2 expression during the early phases of injury in a neonatal rat model of NEC.

LPS (endotoxin) is a bacterial product that has the capacity to produce potent inflammatory responses through the induction of various proinflammatory cytokines such as PAF and TNF. Recent studies suggest that LPS binds with pattern recognition receptors on the intestinal epithelial barrier, such as Toll-like receptors (TLRs), formylated peptide receptors (FPRs), or nucleotide-binding oligomerization domain-like receptors (NODs), to trigger mitogen-activated protein kinase (MAPK), nuclear factor kappa-B (NF-κB), and caspase-dependent pathways, thus leading to various inflammatory responses (Fig. 94-2).[184] Furthermore, Grishin[185] has demonstrated that LPS stimulates p38 MAPK-dependent expression of cyclooxygenase-2 (COX-2) in a rat model of NEC. Systemic injection of LPS has been shown to produce hypotension, shock, and severe intestinal necrosis.[25] It has been used in animal models of NEC to reliably generate intestinal injury that resembles NEC.[186]

## PATHOGENESIS

Despite many years of extensive investigation and the identification of several key risk factors, the precise pathogenesis of NEC remains elusive. The etiology is likely multifactorial and involves a combination of mucosal compromise, pathogenic bacteria, and enteral feedings that in a susceptible host results in bowel injury and an inflammatory cascade (see Fig. 94-2). Of the risk factors, prematurity is the most
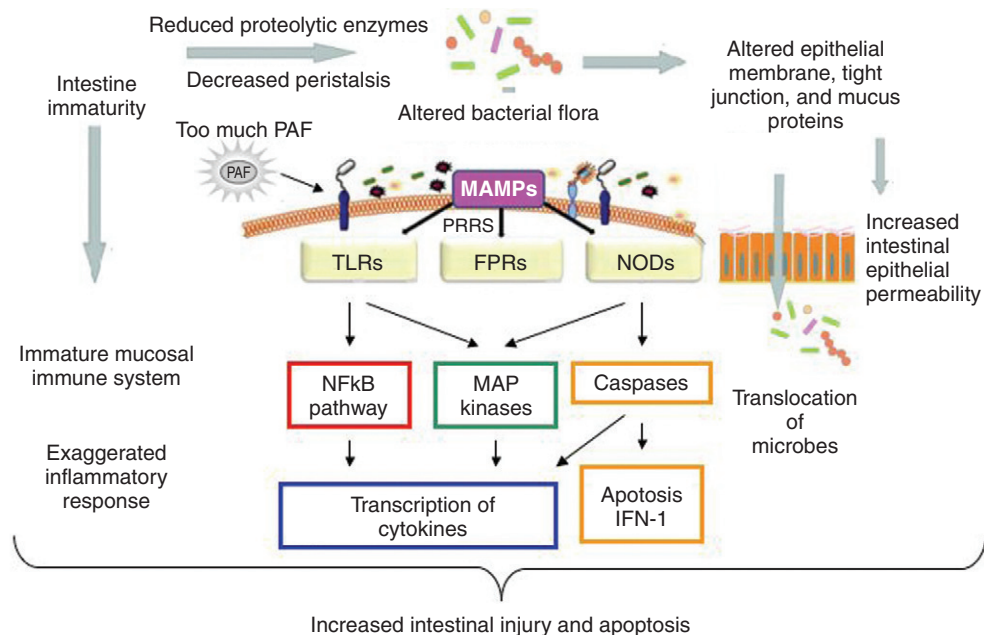


**FIGURE 94-2** Schematic summarizing the pathologic features that contribute to the pathogenesis of necrotizing enterocolitis.

consistent along with the enteral feeding of formula. Commercially available formulas lack many of the beneficial properties present in breast milk. These protective agents include gut trophic hormones, factors that induce intestinal maturation, factors that enhance colonization by nonvirulent bacteria, antiinflammatory mediators, vitamins, antioxidants, and components that support cellular and humoral immunity.[187–190]

## Evidence for an Impaired Gut Barrier

The preterm GI tract is characterized by an immaturity of cellular and humoral immunity,[191,192] increased permeability,[193] reduced gastric acid secretion,[194] reduced concentration of proteolytic enzymes,[195] incomplete innervation and decreased motility,[196,197] and immaturity of the intestinal epithelium and microvilli barrier function.[198] Decreased barrier function is evident in otherwise healthy preterm infants by their ability to systemically absorb and deliver undigested macromolecules, whole bacteria, and LPS.[199–201] Compromise of the intestinal epithelial barrier appears to be the first event leading to activation of the inflammatory cascade.

Reduced mucosal blood flow leading to cellular hypoxia and injury is one of the most frequently cited etiologic factors for NEC. Touloukian[202] emphasized the high incidence of perinatal physiologic stressors that may primarily or secondarily cause intestinal ischemia. Chief among these factors are hypoxic and hypotensive episodes, exchange transfusions through the umbilical vein, umbilical artery catheters, cardiovascular lesions, and serum hyperviscosity. The "diving reflex" in which blood is preferentially diverted away from the splanchnic circulation in order to maintain adequate perfusion of the heart and brain has been hypothesized to occur during these episodes.[203] During periods of low blood flow and subsequent reperfusion, Parks[204] found that a reaction among xanthine oxidase, hypoxanthine, and molecular oxygen results in a burst of superoxide radical production. These free radicals may cause damage to cellular and mitochondrial membranes and alter the permeability of the intestinal mucosal barrier. Although animal studies provide support for this theory, clinical correlation has been lacking, and prospective clinical trials have not been able to consistently establish an association between a hypoxic event and the development of NEC.[205]

Extensive investigation into the critical role of various inflammatory mediators in the pathogenesis of NEC has been conducted. Studies in animal models and human specimens have identified PAF, LPS, NO, and TNF-α as leading potential mediators of NEC.[160,168,169] Increased mucosal permeability and susceptibility allowing translocation of bacteria or bacterial toxin and activation of the inflammatory cascade are thought to be the critical steps leading to the final collapse of intestinal epithelial integrity.[206] Recent studies have suggested that the disruption in the intestinal mucosal barrier results from accelerated apoptosis. All of the key mediators identified in NEC (PAF, NO, LPS, TNF-α) have been shown to cause apoptosis of intestinal epithelial cells.[160,207–209] In a neonatal rat model of NEC involving formula-fed animals exposed to hypoxia and cold stress, Jilling[210] demonstrated a marked increase in apoptosis in the epithelial layer of the NEC group in comparison with mother-fed controls. The accelerated apoptosis was shown to precede gross

morphologic changes in the intestinal epithelium. Administration of a pan-caspase inhibitor to inhibit intestinal apoptosis resulted in a significantly reduced rate of epithelial apoptosis, as well as a decreased incidence of NEC, thus suggesting that apoptosis was the underlying cause of the subsequent mucosal damage, ultimately leading to NEC.

Sustained overproduction of NO secondary to up-regulation of NOS-2 in the GI tract in response to an inflammatory stimulus has been suggested to induce cellular injury and disruption of the intestinal epithelial barrier through a variety of mechanisms.[156] Ford[160] demonstrated up-regulation of NOS-2 and NO in the intestinal wall of infants with NEC and increased apoptosis of enterocytes mediated by the potent oxidant peroxynitrite. This accelerated apoptosis is thought to result in a break in the villus tip of the intestinal mucosal barrier where bacteria may attach, translocate, and initiate an inflammatory cascade.[211] In addition, peroxynitrite has also been shown to inhibit the proliferation of intestinal epithelial cells in a rat model of NEC.[212] The data suggest that in conditions associated with sustained overproduction of NO or peroxynitrite (e.g., NEC), intestinal epithelial barrier dysfunction may result from an imbalance caused by accelerated epithelial injury and blunted tissue repair mechanisms.

## Role of Infectious Agents

The type of feeding and pattern of intestinal colonization may determine the risk for development of NEC. Breast-fed infants become colonized predominantly with bifidobacteria (grampositive bacteria) that help control the growth of gram-negative bacteria.[213,214] In contrast, formula-fed infants become colonized predominantly by coliforms, enterococci, and *Bacteroides* species.[215]

The intestinal mucosa serves as a barrier to the largest microbial challenge to the human body. Beyond serving as a simple physical barrier, the dynamic interaction between the intestinal mucosa and colonizing microbes is an integral part of the innate immune system and is regulated by a system of pattern recognition receptors (PRR). During gut colonization, crosstalk between the system of PRR and commensal bacteria occurs, resulting in intestinal mucosal tolerance or hyporesponsiveness toward the enteric microbes in order to establish a symbiotic relationship. The PRR respond to microbial associated molecular patterns (MAMP) (i.e., LPS, flagellin, peptidoglycans) in order to modulate the cellular response through a variety of downstream signaling pathways (see Fig. 94-2). The PRR system includes the TLRs, which are largely responsible for sampling and interpreting the extracellular environment, and in this capacity, the TLRs have been implicated as an integral mechanism to the pathogenesis of NEC.[184] Hackam and colleagues have shown that the LPS receptor, TLR4, is integral to intestinal mucosal repair in rodent models of NEC through its effects on enterocyte apoptosis and migration.[216] Work by this same group has demonstrated increased expression of TLR4 in the human intestine that develops NEC. There is an intense and ongoing interest in this line of investigation as a primary determinant in establishing a symbiotic gut-microbe axis, which may provide protection against the development of NEC.

The importance of bacteria in the pathogenesis of NEC is supported by the following evidence: (1) NEC occurs in episodic and epidemic waves in which affected patients are

related in place and time or had the same infectious agent[217,218]; (2) during clustered occurrences, the identical microorganisms can be isolated from both afflicted babies and their caretakers[219]; (3) NEC can occur in infants with no known risk factors; (4) NEC can develop several weeks or months after a perinatal insult, when the GI tract is fully colonized and has had sufficient time to recover from any perinatal insult[220,221]; (5) administration of large doses of vitamin E (interferes with intracellular killing of bacteria by leukocytes) to premature infants has been linked with an increase in the incidence of NEC[222]; (6) a NEC-like disease occurs in vulnerable hosts after the ingestion of *Clostridium* species[223]; (7) lesions resembling NEC can be reproduced experimentally with the administration of LPS[169]; (8) endotoxemia is demonstrated in 80% of those with NEC and positive blood cultures for gram-negative bacteria[224]; and (9) pneumatosis intestinalis is a common radiographic finding and represents submucosal gas collections produced by bacterial fermentation.[225,226]

## Unifying Hypothesis for Necrotizing Enterocolitis

Taken together, empiric and experimental data suggest that NEC occurs in a vulnerable host that has become further compromised at the level of the gastrointestinal tract. Infant prematurity and care in the NICU conspire to result in the initiation of bacterial insult or invasion of the immature GI tract, whose key functions including barrier function and immune modulation are altered. As a result, the interaction among the enterocyte, immune effector cells, and resident microbiota initiates an inflammatory cascade that becomes unbalanced, resulting in progressive enteric mucosal injury and increased permeability. In recent years the key role of the gastrointestinal tract as an immune system organ has been increasingly documented.[227,228] Because the microbiota of the human GI tract has increasingly been identified as playing a key role in overall human health through this symbiotic relationship, gut colonization during the newborn period likely presents a particularly vulnerable time for innate immune system, human gut, and gut microbial community dysfunction to occur when this process is disrupted or delayed. The process of gut colonization has been elegantly documented to involve both environmental and genetic factors as evidenced by the findings in twins of similarities in gut microbiota.[229] Moreover, the process is active and dynamic, undergoing a significant shift even in well newborns and infants throughout the first year of life.

## Pathology

NEC may involve single (50%) or multiple (discontinuous) segments of intestine, most commonly in the terminal ileum, followed by the colon.[230] Involvement of both the large and small intestine occurs in 44% of cases. Pan involvement (pannecrosis, NEC totalis) is a fulminant form of NEC characterized by necrosis of at least 75% of the gut, and it accounts for 19% of all cases of surgically treated NEC and most of the deaths.[26]

At surgery, the gross appearance of NEC is fairly constant. The bowel is markedly distended with patchy areas of thinning (Fig. 94-3). The serosal surfaces are typically red to gray and may be covered by a fibrinous exudate. With frank



**FIGURE 94-3** Intraoperative photograph of intestine with necrotizing enterocolitis demonstrating areas of hemorrhagic necrosis and gangrene.

gangrene, the serosal surface is black or, in the most advanced cases, bland gray to white, given the complete loss of perfusion. Subserosal gas collections are frequently encountered. The mucosal surface may be ulcerated with wide areas of epithelial sloughing. Bloody peritoneal fluid is seen when bowel necrosis is present, and brown and turbid fluid is seen when perforation has occurred.

Histologically, enteric inflammation is nearly ubiquitous in NEC (Fig. 94-4). The degree and nature, however, vary from one area to another. Acute and chronic inflammatory changes coexist in 60% of cases. The most common microscopic lesion is bland or coagulation necrosis of the superficial mucosa (89%).[231] Edema and hemorrhage of the submucosa follow complete mucosal necrosis (see Fig. 94-4). Pneumatosis intestinalis is initially seen in the submucosa and later in the muscularis and subserosa. Bacteria in the bowel lumen and wall are present in up to 40% of cases and are occasionally found in gas cysts. Transmural necrosis, characterized by hyaline eosinophilia and loss of nuclear detail in the muscular layers, is present in advanced disease. Epithelial regeneration, formation of granulation tissue, and early fibrosis are often present during the resolution of NEC. This suggests a suppurative process lasting at least several days. Granulation tissue with mucosal and submucosal fibrosis may be seen adjacent to areas of active mucosal and submucosal necrosis and may account, in part, for late stricture formation. Thrombi are sometimes noted in small mesenteric vessels and in small arterioles of the submucosa. Small-vessel thrombosis within necrotic tissue is considered a secondary change. Large-vessel thrombosis is a relatively rare finding at autopsy.

## Diagnosis

### CLINICAL FEATURES

NEC is commonly heralded by nonspecific clinical findings that simply represent physiologic instability.[231,232] These findings include lethargy, temperature instability, recurrent
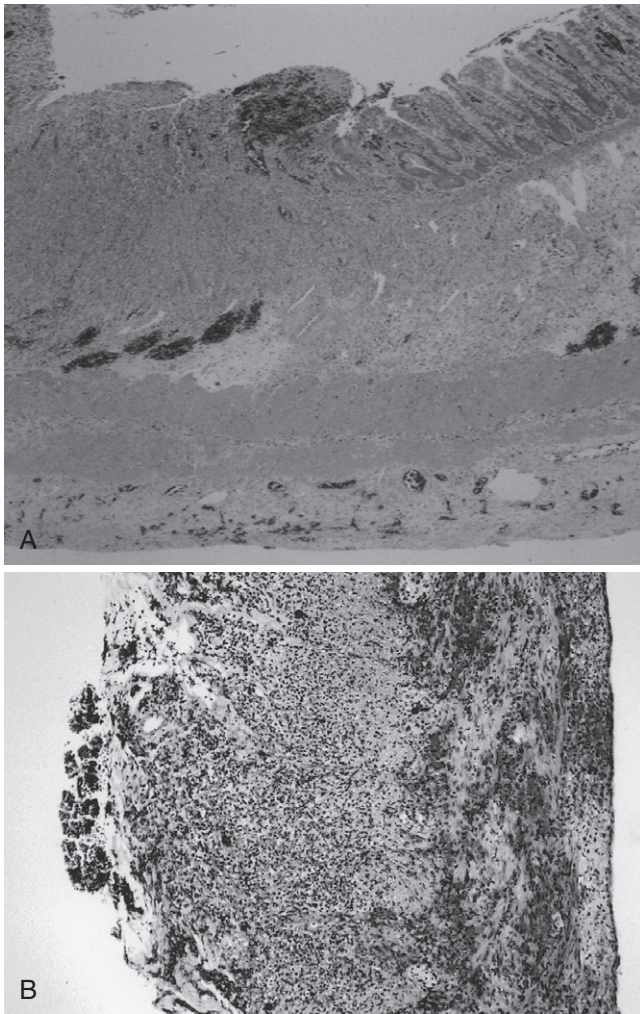
FIGURE 94-4  **A,** Histologic section demonstrating early necrotizing enterocolitis with inflammation and pneumatosis intestinalis in the sub-mucosa (hematoxylin-eosin stain, ×150). **B,** Histologic section demonstrating advanced necrotizing enterocolitis with transmural necrosis and loss of villus and crypt architecture (hematoxylin-eosin stain, ×150).

apnea, bradycardia, hypoglycemia, and shock. More specific symptoms related to the GI tract include abdominal distention (70% to 98%), blood per rectum (79% to 86%), high gastric residuals after feeding (>70%), vomiting (>70%), and diarrhea (4% to 26%). Gross blood in the stool is present in 25% to 63% of cases and occult blood in 22% to 59%. Rectal bleeding is seldom massive. Because the spectrum of disease severity varies, physical examination may initially demonstrate only subtle abdominal distention and minimal tenderness. As the disease progresses, abdominal palpation may elicit tenderness and demonstrate palpable bowel loops, a fixed or mobile mass, or abdominal wall crepitus. Edema and erythema of the abdominal wall as a result of the underlying peritonitis are present initially in approximately 4% of cases but are more common later in the course of the disease. In males, there may be discoloration of the scrotum, indicative of perforation. In a small subset of patients, the disease is rapidly progressive and the initial manifestation is heralded by florid clinical findings and death within 24 hours.

## LABORATORY FINDINGS

Infants with NEC usually have neutropenia, thrombocytopenia, and metabolic acidosis. The total leukocyte count may be elevated, but it is generally low. In one study, 37% of infants had absolute neutrophil counts less than 1500 cells/mm[233]. Infants with the lowest counts in this study had the worst prognosis. Neutrophil counts less than 6000 cells/mm are most commonly associated with concomitant gram-negative septicemia. Some authors[234] have advocated that surgical treatment should be considered in infants older than 34 weeks' gestation if they have lower total neutrophil counts, a higher immature neutrophil number, and a greater immature-to–total neutrophil ratio at first presentation of NEC.

Thrombocytopenia is nearly universally present and seems to be associated with gram-negative sepsis and platelet binding by endotoxin. The incidence of thrombocytopenia in NEC is 65% to 90% and essentially unchanged from the earliest reports from the 1970s.[233,235] O'Neill[236] demonstrated that in a cohort of 40 infants who underwent surgery for NEC, 95% had platelet counts less than 150,000 cells/mm. Rowe and colleagues[237,238] found that platelet counts less than 150,000 cells/mm were present in patients who had positive cultures for gram-negative organisms. The nadir platelet count during the course of the disease was noted to be lower in patients with more severe disease and in those who died.[239] A platelet count less than 104/L or a rapid fall is a poor prognostic indicator. A retrospective single-center study of 91 infants by Kenton[240] suggests that a platelet count less than 104/L within 3 days of the diagnosis of NEC should warrant surgical intervention to decrease the likelihood of bowel gangrene and its attendant morbidity and mortality.

## EMPIRIC AND EXPERIMENTAL INDICATORS OF NEC AND ITS SEVERITY

Metabolic acidosis is common (40% to 85% of patients with NEC) and is believed to result from hypovolemia and sepsis. It is not a specific indicator of intestinal necrosis. Stool samples are commonly positive for occult blood and reducing substances. Book[241] reasoned that intestinal mucosal damage from NEC leads to carbohydrate malabsorption. Poorly digested carbohydrates pass into the colon, where they are fermented and excreted in stool. The authors tested the stool of formula-fed infants for reducing substances with reagent (Clinitest) tablets and found that 71% of formula-fed infants in whom NEC developed had greater than 2+ reducing substances in their stool. Colonic bacterial fermentation increases the local production of D-lactate, which is absorbed and excreted by the kidneys. Garcia[242] could show elevated urinary D-lactate levels in infants with NEC but not in control infants. With recovery from NEC or administration of enteral antibiotics, D-lactate excretion decreased. Similarly, hydrogen excretion in the breath is elevated when fermentation is increased. This test is helpful in ruling out NEC. A negative result on a breath hydrogen test is 99% accurate in ruling out NEC.[243] Abubacker[244] indicated via a study of 24 infants with NEC that a preoperative blood lactate level of greater than 1.6 mmol/L carries a poor prognosis with mortality odds ratio of 22 (CI 1.54 to 314.3, $P = 0.04$). Finally, a multicenter study of 473 infants with NEC by Moss and colleagues[245] identified metabolic acidosis

at diagnosis (pH < 7.3 or bicarbonate < 16) as one of the 12 parameters that may assist in predicting the progression of NEC.

In an uncontrolled retrospective study comparing two centers' criteria for surgical intervention, Tepas[246,247] found that if three of seven critical metabolic derangements (positive blood culture within 96 hours of diagnosis, pH < 7.25 or receiving bicarbonate, bandemia > 20%, serum sodium < 130 mEq/L, platelet count < 50,000 cells/mm$^3$, MAP < gestational age or on any pressors, and absolute neutrophil count < 2000) were identified, this permitted earlier identification of infants needing exploration and resulted in better surgical outcomes (mortality and the need for postoperative parental nutrition) than did delaying operation until radiographic proven evidence of perforation by pneumoperitoneum.

C-reactive protein (CRP), an acute phase reactant, has been measured in an attempt to correlate its level with the presence, absence, or resolution of the disease.[248] CRP may serve as an early indicator of NEC when levels rise more than 10 mg/L within 48 hours of the suspected diagnosis (reported sensitivity, 92%; specificity, 81%). Failure of CRP to return to normal within 10 days was an indicator of abscess, stricture, or septicemia. A systematic review of various biomarkers for NEC[56] selected six qualified studies[248–253] on CRP that together indicated CRP is a relatively sensitive but nonspecific marker for NEC, yielding a combined diagnostic odds ratio (DOR) of 5.82 and an area under the curve (AUC) of 0.70. The diagnostic odds ratio expresses how much greater the odds of having the disease are for people with a positive test result rather than for the people with a negative test result. In this case, although the DOR is high, its AUC is only 70%, implying a weaker diagnostic performance.

*Intestinal fatty acid–binding protein* (I-FABP) is a small (14 to 15 kd) cytoplasmic protein of mature enterocytes. It is released into the circulation upon death of enterocytes, thus representing intestinal injury when detected in high concentration. Lieberman,[254] Edelson,[255] and Guthmann[256] have demonstrated an elevated level of I-FABP in the serum of neonates with NEC. Given its small size and its ability to pass through the glomerulus, urinary I-FABP has been detected as potential marker of NEC by Derikx.[257] Evennett[258] further characterized the release of urinary I-FABP by correlating its I-FABP-to-creatinine concentration with the severity of NEC. The I-FABP-to-creatinine concentration was significantly higher in infants with extensive disease than in those with focal disease (7.4 pg/mmol [2.1 to 35 pg/mmol] vs. 1.1 pg/mmol [0.3 to 1.7 pg/mmol], respectively, $P = .002$). Although studies have shown higher concentrations of I-FABP in infants with severe NEC, Thuijls[259] concluded urinary I-FABP not to be an effective screening tool for NEC given its ability to identify merely one third of neonates with NEC in a group of 226 neonates with no clinical suspicion of NEC originally. This study was performed on the basis of a cutoff point of 2.20 pg/nmol creatinine derived from testing urinary I-FABP on 35 neonates suspected of NEC. However, Thuijls[259] repeated the conclusion by Evennett[258] that urinary I-FABP is a promising prognostic marker for NEC.

Calprotectin, a calcium-binding protein found in neutrophils and macrophages, is a marker of inflammation of the gastrointestinal tract. It has been found in stool due to transepithelial migration of myeloid cells and has been a useful marker for exacerbation of inflammatory bowel disease in children.[260] Josefsson found fecal calprotectin to be elevated greater than 2000 µg/g feces in NEC with perforation with microscopic bowel inflammation but less than 2000 in cases with focal disease. Thus similar to I-FABP, it may be a useful marker for disease severity but not a strong screening tool.

# Microbiology

It has proven extremely difficult to identify common offending infectious agents in infants with NEC. Organisms recovered from the blood and stool of patients with NEC vary depending on the GI tract flora, the nosocomial flora, the site cultured, and the duration of previous antibiotic therapy. It is unclear whether the bacteria cultured represent pathogens causing NEC or, instead, secondary opportunistic invaders selected by the antibiotic regimen. Furthermore, lack of sufficiently matched controls and incomplete bacteriology data on other patients in similar environments makes study of the microbiology of NEC difficult.

## BACTERIOLOGY

Bacteriologic data for NEC have primarily been based on cultures obtained from the blood, stool, and peritoneal cavity. Blood cultures are positive in 30% to 35% of patients.[215] Cultures commonly grow *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis,* enterococci, *Clostridium perfringens,* and *Pseudomonas aeruginosa. K. pneumoniae* and *E. coli* cause the majority of positive blood cultures. The organisms most frequently cultured from stool specimens are *E. coli, K. pneumoniae, Enterococcus cloacae, P. aeruginosa, Salmonella species,* coagulase-negative staphylococci (*S. epidermidis*), *C. perfringens, Clostridium difficile,* and *Clostridium butyricum.*[261–263] Peritoneal cultures most commonly grow *Klebsiella species, E. coli,* coagulase-negative staphylococci, *Enterobacter* species, and yeast.[264]

## FUNGAL CULTURES

In contrast to bacteria, no data implicate a fungus as an initiating organism in the pathogenesis of NEC. Fungi are believed to be secondary invaders. Fungal septicemia with *Candida* species has been implicated in many late NEC deaths.[265,266] In a retrospective premortem and postmortem examination of body fluid and tissue cultures from 30 patients who died of NEC by Smith and colleagues,[265] 47% of patients had evidence of fungal infection and colonization was found in 20%.

## CLUSTERED EPIDEMICS

In most large series, sporadic cases have been followed by the sudden appearance of a cluster of a relatively large number of cases. In many of the epidemics, no specific pathogen has been identified. During these episodes, nursery personnel experienced concomitant acute GI illnesses.[21,267] In those few studies in which a specific pathogen is identified, it was in association with documented contamination of milk formula or milk fortifier that was used in the NICU.[268] Identified species have

included *E. coli, Salmonella species, C. difficile,* rotavirus, noro-virus, torovirus, and a coronavirus-like organism.[217,268–274]

# Imaging

The cornerstone of the diagnosis of NEC remains plain ante-roposterior and left lateral decubitus radiographs. Any or all of the following findings are associated with NEC: ileus pattern (nonspecific bowel distention), pneumatosis intestinalis (linear or cystic) (see Fig. 94-1), portal vein gas, pneumoperitoneum, intraperitoneal fluid, and persistently dilated, fixed loops.[275] Both pneumatosis and portal vein gas are often fleeting signs but are considered pathognomonic in the appropriate clinical setting of prematurity. In more recent years, there has been a trend and tendency toward the increased use of bedside ultrasound with Doppler imaging of the GI tract in an effort to identify more subtle findings like abdominal fluid, hyperemia, decreased blood flow to the gut, and pneumatosis intestinalis, all of which may not be well seen by plain radiograph.[276–291] Despite this trend, to date there have been few good comparative studies documenting the clinical utility of ultrasound in the diagnosis of NEC.

## BOWEL DISTENTION

Multiple gas-filled loops of intestine are the earliest and most common radiologic finding in patients with NEC (55% to 100% of cases).[292] As fluid and air accumulate, air-fluid levels are visible on the decubitus view. The degree of dilatation and the distribution of bowel loops are related to the clinical severity and progression of the disease. In some cases, nonspecific intestinal dilatation precedes clinical symptoms suggestive of NEC by several hours.

## PNEUMATOSIS INTESTINALIS

Demonstration of pneumatosis intestinalis (intramural gas) in the appropriate clinical setting is diagnostic of NEC (see Fig. 94-1). The air mainly comprises hydrogen, a byproduct of bacterial metabolism. The frequency of pneumatosis intestinalis ranges from 19% to 98%, although it may be absent in up to 14% of patients with NEC (even severe disease).[205] Conversely, extensive pneumatosis may be present with minimal signs; it often responds promptly to medical management. Pneumatosis is fleeting, may appear before the onset of clinical symptoms, and is commonly an early rather than a late finding. Pneumatosis is most frequently noted when infants have been fed (84%), in contrast to unfed babies (14%).[31] Pneumatosis intestinalis is not specific for NEC and has been noted in infants with enterocolitis of Hirsch-sprung disease, inspissated milk syndrome, pyloric stenosis, severe diarrhea, carbohydrate intolerance, and other disorders. Two forms of pneumatosis intestinalis are recognized radiographically: cystic and linear. The cystic form has a granular or foamy appearance and represents gas in the submucosa. It is often confused with fecal material in the large intestine. Linear pneumatosis consists of small bubbles collected within the muscularis and subserosa to form a thin linear or curvilinear gas pattern outlining the wall of a segment of the intestine.

## PORTAL VEIN GAS

Portal vein gas (PVG) appears as linear branching radiolucencies overlying the liver and often extending to its periphery (see Fig. 94-1). It represents gas dispersed through the fine radicles of the portal venous system. The presence of portal vein gas is fleeting, which perhaps accounts for the low reported incidence of 10% to 30%.[293] In most series the presence of portal vein gas is associated with a poor prognosis.[205] In cases with pan-intestinal involvement, PVG is present in 61% of patients. The genesis of portal vein gas may involve accumulation of gas in the bowel wall as a result of bacterial invasion, dissection into the venous system, and migration to the radicles of the portal vein. Alternatively, it may represent the action of gas-forming bacteria within the portal venous system itself.

## PNEUMOPERITONEUM

Free air in the peritoneal cavity associated with perforation of the intestine can be demonstrated in 12% to 30% of patients. It is best noted on the left lateral decubitus or cross-table lateral view. Upright radiography is unnecessary. A supine view of the abdomen can demonstrate free air by outlining the falciform ligament ("football sign"), the umbilical artery, or urachal remnants or by revealing the "double-wall" sign. This sign refers to visualization of air on both sides of the wall (lumen and peritoneal cavity). In patients who have intestinal perforation proven by surgery, radiographic evidence shows free air in only 63%, thus demonstrating that perforation can occur in a surprisingly high number of patients without evidence of pneumoperitoneum. One must also recall that pneumoperitoneum may occur without intestinal perforation from mechanical ventilation for severe lung disease. In this clinical situation, barotrauma may produce alveolar rupture with air dissection into the abdomen through the mediastinum. The patient's signs, symptoms, and laboratory findings will often differentiate the cause of the air. If one is unsure, abdominal paracentesis may be performed and any aspirated fluid analyzed. If there is no ascites, a water-soluble contrast study via the gastric tube may be performed to rule out gastric perforation.

## INTRAPERITONEAL FLUID

Several plain radiographic findings suggest free fluid in the peritoneal cavity that is amenable to paracentesis: (1) a grossly distended abdomen devoid of gas, (2) gas-filled loops of bowel in the center of the abdomen surrounded by opacity out to the flanks, (3) increased haziness within the abdomen, and (4) separation of bowel loops. These findings have been reported in 11% of cases. Both ascites and portal vein gas are radiographic findings associated with high mortality rates. Twenty-one percent of patients with surgically proven intestinal perforation have ascites. However, 16% of all patients with proven intestinal perforation have neither ascites nor pneumoperitoneum on plain radiographs.

## PERSISTENT DILATED LOOPS

The "persistent dilated loop sign" is a plain radiographic finding that was described by Wexler[294] in a study of five babies with NEC in whom a single loop or several loops of dilated bowel remained unchanged in position and configuration for 24 to 36 hours. Full-thickness necrosis subsequently developed in these patients. This finding, however, does not

always indicate bowel necrosis. Leonard[295] found a persistent loop in 33% of 21 patients with proven NEC. Fifty-seven percent of infants with a persistent loop had necrotic intestine at surgery or autopsy, but necrosis never developed in 43% and they recovered with nonoperative treatment.

## CONTRAST STUDIES

Radiopaque contrast studies of the upper GI tract may occasionally be useful to improve diagnostic accuracy in patients with equivocal clinical and radiologic signs of NEC. However, overdiagnosis by contrast radiography is possible and may lead to unwarranted treatment. Careful attention to the type of contrast agent used for the study is critical.[294] Barium should never be used because extravasation of a barium and stool mixture through a perforation may intensify the peritonitis. Unlike barium, water-soluble contrast agents are absorbed by both the bowel and the peritoneal cavity. The practical implication of this absorption is a transient (6 to 12 hours) increase in urinary specific gravity. Historically, water-soluble agents were hyperosmolar and caused dangerously large intraluminal fluid shifts, especially in premature patients. Current water-soluble agents are non-ionic, have much lower osmolarity, and produce excellent opacification of the GI tract. NEC is suspected when intestinal contrast enhancement demonstrates bowel wall loops separated by edematous walls, an irregular mucosa with ill-defined margins, mucosal ulceration, bowel wall spiculation, or pneumatosis intestinalis. Currently, the use of contrast studies is usually reserved for interrogation of the GI tract after the acuity of NEC has resolved and in order to examine for stricture formation during or after recovery. Though advocated by some,[296] contrast enemas should not be performed because of the risk for rectosigmoid perforation. Unless there is colonic disease or reflux of contrast into a diseased distal ileum, the contrast enema will not be diagnostic of NEC. It may, in fact, overdiagnose NEC because contrast enemas have been shown to produce pneumatosis and transient portal venous air. In an infant who has recently completed a course of therapy for NEC but has persistent signs of partial small bowel obstruction or blood-tinged stools, contrast enemas may be useful in identifying strictures.

## ULTRASONOGRAPHY

Ultrasonography (US) has been used to identify necrotic bowel, intraperitoneal fluid, and portal venous air. However, abdominal sonography can depict the following findings highly suggestive of nonviable bowel over plain abdominal radiography: presence of intra-abdominal fluid, thinning of the bowel wall, reduction of bowel wall perfusion, abnormal bowel loops, and intermittent gas bubbles in liver parenchyma and the portal venous system.[297] The abnormal bowel loops on US are characterized by a hypoechoic rim with a central echogenic focus ("target sign").[278] Appearing as pericholecystic hyperechogenicity, the intermittent gas bubbles in liver parenchyma and the portal venous system are believed to represent either pericholecystic venous gas or extension of the foamy inflammatory infiltrate of NEC into the pericholecystic space.[298,299]

Theoretically, US may have significant value if it can identify patients who require operation in a more sensitive and timely manner than is otherwise possible with conventional clinical and radiographic methods. Although Silver[299] has correlated seven sonographic findings with adverse outcomes needing surgical intervention, more studies are necessary to compare the sensitivity and specificity, as well as intraobservable and interobservable agreements between sonographic and radiographic findings. Thus at this time, the use of US for the diagnosis of NEC is most applicable to patients with questionable clinical and radiologic findings or to localize intra-abdominal fluid for paracentesis.

## MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) is a noninvasive modality that has recently been used to identify infants with ischemic bowel secondary to NEC. Although MRI is capable of demonstrating the cardinal findings of NEC, its utility is limited.[300]

# Classification

To select the appropriate treatment (nonoperative vs. operative) and to determine the impact of therapy on survival and late outcomes, it is essential that investigators use comparable criteria for classifying the stages of NEC. Several classification schemes have been proposed. In 1978 Bell[8] introduced the now most commonly used three-stage classification system (suspected, definite, and advanced) that categorizes patients by historical factors, GI manifestations, radiologic findings, and systemic signs (see Table 94-1). The Bell staging criteria have been modified[220]; the three stages are still used, but subsets are included in an effort to identify specific prognostic factors. Infants with stage I disease have features suggestive of NEC, patients with stage II disease have definitive NEC without an indication for surgical intervention, and patients with stage III disease have advanced NEC with evidence of bowel necrosis or perforation.

# Management

## NONOPERATIVE

In the absence of intestinal necrosis or perforation, the mainstay of treatment for patients with NEC is supportive. Feedings are discontinued, the GI tract is decompressed through a sump gastric tube, and intravenous fluid resuscitation is initiated. A complete blood count, platelet count, blood gas analysis, and CRP and serum electrolyte levels are obtained. Blood and urine samples are sent for culture, and broad-spectrum intravenous antibiotic therapy is initiated. Until recently, most antibiotic regimens included a penicillin, an aminoglycoside, and an agent effective against anaerobic organisms. It seems logical that coverage for anaerobic organisms be included because these infants are usually 1 to 2 weeks old and have or are undergoing colonization by coliforms. To date, no controlled study has shown the efficacy of this regimen. The antibiotic regimen is best tailored not only to the most common organisms found with NEC but also to the nosocomial nursery flora. Because of recent reports of patients with stool and blood cultures positive for coagulase-negative staphylococci, some groups now empirically treat patients with a combination of vancomycin and gentamicin or vancomycin and a third-generation cephalosporin.[301] The incidence of fungal sepsis in infants who die of NEC is high, so a strong index of suspicion must be maintained and empirical antifungal therapy should be considered if the patient's clinical course is prolonged. Historically, close clinical observation consists of frequent physical examination, two-view abdominal radiography

performed every 6 to 8 hours, serum platelet and leukocyte counts, and blood gas analysis. However, one could question the utility of the frequent use of plain radiographs at this interval, given the number of cases that have progressive NEC without pneumoperitoneum and depending on the individual practitioners' trigger for operative intervention.

Patients with definite disease of moderate severity (Bell stage II) are normally treated by bowel rest, decompression, and antibiotic therapy for at least 7 to 14 days. A central venous catheter may be placed for total parenteral nutrition. If the patient is clinically well, small amounts of formula may be restarted. The infant is constantly and carefully monitored for abdominal distention, vomiting, or nonspecific signs or symptoms of NEC. Once feedings resume, stools are tested for reducing substances and occult blood. Feedings are discontinued if the result of either test becomes positive. Infants who have undergone surgery receive 1 to 2 weeks of postoperative intravenous antibiotics. Feedings are initiated when the patient is clinically well and return of bowel function has been established.

## INDICATIONS FOR OPERATION

The principal goals of surgical intervention in the setting of NEC are to remove gangrenous bowel and preserve intestinal length.[302,303] On the basis of historical clinical experience, many argue that exploration should not be undertaken until gangrene is present but should be performed before perforation occurs. Unfortunately, no combination of clinical examination or adjunct testing has been shown to have high sensitivity for intestinal gangrene.[304,305] Thus there remains controversy regarding the indications for operation, the most appropriate timing of intervention, and the optimal surgical treatment strategy. The most widely accepted indication is the presence of pneumoperitoneum. Relative indications include a positive paracentesis, palpable abdominal mass, abdominal wall erythema, portal venous gas, fixed intestinal loop, and clinical deterioration despite maximal medical therapy.

In an attempt to identify characteristics that may serve as predictors of intestinal gangrene, Kosloske[304] reviewed 12 criteria used as indications for operation in 147 patients with NEC and stratified these criteria according to sensitivity, specificity, positive/negative predictive value, and prevalence. The "best" indicators (specificity and positive predictive value [PPV] approaching 100%, prevalence > 10%) were pneumoperitoneum, positive paracentesis (aspiration of > 0.5 mL brown or yellow fluid containing bacteria on Gram stain), and portal venous gas. "Good" indicators (specificity and PPV approaching 100%, prevalence < 10%) were a fixed intestinal loop, erythema of the abdominal wall, and a palpable abdominal mass. A "fair" indicator (specificity of 91%, PPV of 94%, prevalence of 20%) was "severe" pneumatosis intestinalis as graded by a radiographic system. "Poorer" indicators were clinical deterioration (PPV of 78%), platelet count lower than 100,000/mm$^3$ (PPV of 50%), abdominal tenderness (PPV of 58%), severe GI hemorrhage (PPV of 50%), and gasless abdomen with ascites (0%). Unfortunately, none of the indicators had sensitivity greater than 48%.

Instead of looking at indicators separately, Coursey[306] combined various radiologic findings into a 10-point Duke Abdominal Assessment Scale (DAAS) to improve intraobserver and interobserver agreement on diagnosing severe NEC. A subsequent validation study by the same authors[307] demonstrated that for every one-point increase in the DAAS score (AUC = 0.83), infants with NEC were significantly more likely to need surgical intervention. Although the study did not define a specific score cutoff for surgical intervention, 93% of the operated infants in this study had a score of 7 or above.

### Pneumoperitoneum

Infants in whom pneumoperitoneum develops during the management of NEC should undergo either laparotomy or peritoneal drain placement. Unfortunately, pneumoperitoneum is not always demonstrable in neonates with gut perforation, with one study reporting that only 63% of infants with perforation demonstrated free air.[308]

### Paracentesis

A positive result on paracentesis, defined as free-flowing aspiration of more than 0.5 mL of brown or yellow-brown fluid that contains bacteria on Gram stain,[156] is highly specific for intestinal necrosis. A negative result on paracentesis is rare with intestinal necrosis but can occur when a localized, walled-off perforation is present or a segment of bowel is injured but not perforated.[309] Currently, there is no absolute indication for paracentesis. Kosloske[304] recommends abdominal paracentesis for patients with extensive pneumatosis intestinalis or for those who do not improve with nonoperative management. If no peritoneal fluid is aspirated, peritoneal lavage is performed by instilling up to 30 mL/kg of normal saline solution into the peritoneal cavity, turning the patient from side to side, and then withdrawing the fluid. Ricketts and Jerles[309] reported a greater than 70% survival rate when a positive result on abdominal paracentesis was used as the indication for exploration in 51% of their patients; three false-negatives occurred. They performed paracentesis when there was erythema and edema of the abdominal wall, portal vein gas, a fixed and dilated loop on sequential abdominal radiography, a fixed and tender abdominal mass, or persistent clinical deterioration. The indications used for paracentesis in this report are considered indications for operation by many surgeons.

### Portal Venous Gas

To determine the significance of portal vein gas in relation to the presence and extent of bowel necrosis and mortality, Kurkchubasche[310] reviewed the experience of Children's Hospital of Pittsburgh, as well as the world literature. Of the 616 patients collected, 118 (19%) had portal vein gas on plain radiography. Of these 118 patients, 102 underwent operation, usually 24 hours after the radiographic appearance of portal vein gas. All 102 patients had full-thickness bowel necrosis, and 52% had necrosis of more than 75% of the length of the entire small intestine. The overall surgical mortality rate for patients with portal vein gas was 52%, and more than 90% of those with pan involvement died. Of the 15 patients who had portal vein gas and did not undergo immediate exploration, bowel necrosis requiring subsequent operation developed in 6 (40%) and 5 of the 6 died. In a separate study, Rowe[26] suggested that intestinal necrosis will develop in more than 90% of infants with portal venous gas, with pan involvement developing in 52%. More recently, in a review of 40 infants with NEC and portal venous gas,

Molik[311] reported a 54% overall operative mortality and pan involvement in 25%.

### Fixed Persistent Intestinal Loop

A fixed dilated intestinal loop is defined by persistent location and configuration for more than 24 hours. In a recent study, approximately half the patients with evidence of a fixed dilated loop recover without an operation.[312] The finding of a fixed loop is considered concerning by most observers as indicating a pregangrenous or severely compromised intestinal loop, yet to date there is little objective evidence to support that assumption. Moreover, at this time there is no general consensus on the utility of fixed loop in guiding operative management.

### Clinical Deterioration

Many criteria for operating on children who show continued clinical deterioration despite adequate supportive therapy exist,[313] but none by themselves are absolute indications. These criteria include abdominal wall erythema, peritonitis on physical examination, persistent/increasing acidosis, and persistent/progressive thrombocytopenia.

### Ascites

As mentioned, pneumoperitoneum may not always be evident with intestinal perforation. A gasless abdomen, suggestive of a fluid-filled abdominal cavity, may be the only indication of perforation. Frey[308] reported that in 21% of infants with intestinal perforation, the only radiographic evidence was ascites. They noted that radiographs are imprecise and that evidence of ascites in the appropriate clinical setting of NEC mandates paracentesis for further evaluation.

## OPERATIVE MANAGEMENT

Advanced disease requiring surgical intervention develops in up to 50% of infants with NEC.[314,315] Until recently, given the lack of quality prospective randomized trials, the optimal surgical treatment strategy for NEC remained controversial. Surgical goals are to remove gangrenous bowel and preserve intestinal length. Within this context, a number of different options exist, but most agree that the surgical approach should be determined by the extent of intestinal involvement. The patient's general condition should be optimized before operation with aggressive ventilatory support, treatment of shock, administration of broad-spectrum antibiotics, and correction of anemia and coagulopathy. Operative procedures may be performed in the NICU under appropriate conditions without an increase in complications.[316]

### Primary Peritoneal Drainage

Treatment of intestinal perforation in VLBW infants remains controversial. In 1977 Ein[317] reported the use of peritoneal drainage (PD) as a means of stabilizing and improving the systemic status of premature infants with perforation before laparotomy. Since then, primary treatment with PD has been used in a variety of settings, and some investigators have suggested that it may serve as definitive therapy. More recently, several studies have attempted to address this issue more rigorously.[318–333] Currently, most surgeons propose PD as the initial treatment in ELBW infants (<1000 g at birth) with perforated NEC to allow resuscitation and stabilization before

definitive laparotomy. In an attempt to address this issue, two multicenter prospective randomized controlled trials were conducted. In the NECSTEPS trial of 117 infants (<34 weeks' gestation and < 1500 g) by Moss,[324] 19 of 55 infants with PD died (34.5%), as compared with 22 of 62 infants with laparotomy (LAP) (35.5%, $P = 0.92$) by 90 days postoperatively. The percentages of infants who became dependent on total parenteral nutrition were 17 of 36 (47.2%) in the PD group and 16 of 40 (40%) in the laparotomy group ($P = 0.53$). Although this study was originally designed to detect a reduction in 90-day mortality from 50% to 25% with a statistical power of 82%, it failed to enroll the desired number of study subjects in the time allotted to the study, thus reducing the power to 77% or less.

Three primary conclusions were drawn from this prospective randomized trial. The trial found that the type of operation performed for intestinal perforation in infants with NEC (1) does not significantly affect 90-day mortality, (2) does not affect rate of dependence on total parenteral nutrition at 90 days postintervention, and (3) did not affect the total length of hospital stay. The results of this trial have led to a follow-up study that seeks to determine the difference in median to long-term outcomes comparing the two surgical procedures with an emphasis on neurodevelopmental outcomes. Two years following the publication of the NECSTEPS trial results, Rees[323] published the results from the European NET trial that included 69 patients. Similar to the results in the NECSTEPS trial, there was no significant difference in the 6-month survival rate between infants undergoing either laparotomy or PD as primary treatment for perforated NEC. Six-month survival was 18 of 35 (51.4%) with PD and 21 of 33 (63.6%) with LAP ($P = 0.3$; Fisher exact text, difference 12%, Cl, (11, 34%). Cox regression analysis showed no significant difference between groups (hazard ratio for PD 1.6; $P = 0.3$; 95% CI, 0.7 to 3.4). However, in the PD group, delayed laparotomy was performed in 26 of 35 (74%) patients after a median of 2.5 days (range, 0.4 to 21) and did not improve 6-month survival compared with primary laparotomy (relative risk of mortality 1.4; $P = 0.4$; 95% CI, 0.6 to 3.4). Unlike the conclusion derived by the NECSTEPS trial, Rees concluded that PD is ineffective as either a temporizing measure or definitive treatment because of the high percentage of infants requiring "rescue" laparotomy.

Even though the two prospective studies did not demonstrate statistically significant differences in mortality between PD and LAP, the most recent meta-analysis by Sola[334] (273 PD, 250 LAP), which selected two clinical trials and three prospective cohort studies[325,326] out of the 12 studies reviewed, indicated an increased mortality of 55% with PD (OR 1.55, 95% CI, 1.08 to .22, $P = 0.02$) without statistical heterogeneity. Upon careful review, four of the five studies showed mortality rates of PD to be higher than LAP and the authors attributed the higher rates to more premature and smaller infants selected for the PD group. Thus controversies persist in initiating PD for stabilization before laparotomy. Currently, in an effort to address intermediate and long-term morbidity, a multicenter trial sponsored by the NICHD Neonatal Research Network was initiated in 2010. The primary objective of this trial is to determine the rate of primarily neurodevelopmental impairment at 2 years of age in ELBW infants with NEC who undergo either PD or laparotomy as primary treatment of perforated NEC.

## Laparotomy

At laparotomy, the extent of NEC may be classified as focal, multifocal, or pan-intestinal (<25% viable bowel). Depending on the extent of disease and patient characteristics at the time of surgery, a number of different surgical options may be undertaken including resection with enterostomy, resection with anastomosis, proximal enterostomy, the "clip-and-drop" technique, and the "patch, drain, and wait" technique. The abdomen is entered via a transverse supraumbilical incision with precautions taken to not injure the liver. Optionally, samples of peritoneal fluid may be harvested for culture of aerobic, anaerobic, and fungal organisms. The entire GI tract is systematically examined to assess the extent of disease and viability of the bowel. If clear demarcation of gangrenous bowel is encountered, it is resected. Marginally viable bowel can be preserved yet defunctionalized either through a proximal diversion (enterostomy) or clip-and-drop technique wherein the intestine is closed at either end using hemoclips in an effort to prevent further passage or leakage of the fecal stream. These latter techniques are particularly useful if the overall length of involved intestine is such that the infant would be rendered "short-gut" if all marginally involved intestinal length is removed. A second-look operative approach to extensive NEC involvement is currently not widely practiced. Infants tend to be quite ill with significant ongoing physiologic instability following most operative interventions for NEC. At the conclusion of the procedure, one should record the length of viable intestine remaining and note the presence or absence of the ileocecal valve.

A rarely cited complication of laparotomy for NEC is spontaneous intraoperative liver hemorrhage from injury caused by retractors or finger dissection. VanderKolk[335] reported this complication in 11.8% of operations for NEC over a 5-year period. The mean gestational age of those with liver hemorrhage was 28 weeks (mean weight, 1262 g). Only one of these patients survived. The authors identified low mean preoperative arterial pressure and high preoperative fluid administration over the preceding 24 hours as significant predisposing factors. This complication occurred shortly after the abdomen was opened, and the intestine was eviscerated. Liver congestion was followed by subcapsular hematoma and then free rupture.

## Focal Disease

When a single area of bowel is necrotic or perforated, only limited resection is necessary. Creation of a proximal enterostomy and distal mucus fistula has been the standard of care. Stomas can be created either through the ends of the incision or through a separate exit site on the abdomen. Factors to be considered when deciding on optimized stoma sites include mesentery that has been shortened by inflammation that may impede exteriorization, placement and fitting of future stoma bags without leakage, ease of future closure if diverting ends are placed in close proximity or remotely, and incidence of additional complications including wound infection and stoma prolapse. Most enterostomies are created by suturing the intestine to the fascia with interrupted sutures. About 2 cm of bowel are left protruding from the abdominal wall, and no attempt is made to "mature" the end of the intestine. If stoma viability is in question postoperatively, a small portion of the full thickness of the intestine is excised at the bedside and the cut ends are observed for bleeding.

Resection with primary anastomosis for isolated disease may be performed in carefully selected patients. Proponents of primary anastomosis cite the high morbidity associated with enterostomies in infants and no need for a second operation.[336] To safely perform this technique, the following criteria must be met: (1) a sharply localized, usually proximal segment of disease; (2) undamaged appearance of the remaining intestine; and (3) stable overall patient physiology without evidence of rapidly progressive sepsis or coagulopathy.

## Multisegmental Disease (>50% Viable)

If the patient has multiple areas of necrosis separated by viable bowel, several options are available. Historically, the surgeon excises each diseased segment individually and creates multiple stomas rather than performing a massive resection. Conversely, a single high stoma (proximal jejunum) may be created and the distal bowel "spliced" together, thereby avoiding multiple stomas. A proximal jejunostomy can cause significant fluid and electrolyte loss and peristomal skin complications, although aggressive skin care with measurement and replacement of stoma losses can avoid these potential complications. Anastomotic strictures are not uncommon and are addressed at the time of jejunostomy closure. Resection plus anastomosis has also gained increased acceptance as a valid treatment option for severe NEC and for multifocal disease.[303] In a study involving 46 infants with multifocal NEC, Fasoli[302] reported a higher survival rate after resection and primary anastomosis (85%) versus enterostomy (50%).

Moore[337] described a controversial approach termed the "patch, drain, and wait" procedure in 1989. The principles of this potentially bowel length–preserving method are transverse single-layer suture approximation of perforations (patch), insertion of two Penrose drains that exit in the lower quadrants (drain), and a commitment to long-term parenteral nutrition (wait). The Penrose drains capture fecal fistulas and function as de facto enterostomies as the peritoneal cavity is rapidly obliterated by adhesions.[338] This procedure does not address the issue of ongoing sepsis because necrotic bowel is not resected, the general peritoneal cavity is difficult to drain, and the thin-walled perforated bowel often cannot handle suture.

Vaughan[339] described a promising novel technique aimed at avoiding multiple enterostomies, circumventing the complications of a high jejunostomy, and preserving bowel length. The authors performed the "clip-and-drop-back" technique in three patients with NEC. In this procedure the obviously necrotic bowel is removed, and the cut ends are closed with titanium clips or staples. Reexploration is performed 48 to 72 hours later, the clips are removed, and all segments are reanastomosed without any stomas. In one of the three patients, reresection was required during the second-look operation, the bowel ends were clipped again, and a successful primary anastomosis was performed during a third operation. Follow-up for this small series was 6 months to 7 years with no anastomotic complications or delayed reoperations.

## Pan Involvement (NEC Totalis, <25% Viable)

Pan involvement develops in 19% of patients[310]; it poses an enormous treatment problem and remains a highly controversial management issue. The overriding consideration is to

spare as much intestine as possible. Treatment options include resection of all necrotic bowel with placement of proximal or multiple stomas or proximal diversion without bowel resection, with plans for a second-look procedure. The decision to forego any treatment is supported by studies that demonstrate a 42% to 100% mortality rate in patients with pan involvement, with almost all survivors left with short-bowel syndrome. The mortality rate is nearly 100% for infants who weigh less than 1000 g.

Diverting the intestinal stream by high proximal jejunostomy (without bowel resection) may facilitate healing of injured bowel through distal intestinal decompression, a reduction in its metabolic demands, and a decrease in the number of bacteria and possibly their byproducts. This technique was initially reported by Martin and Neblett[340] and involves performing a high jejunostomy without resection, with plans for a second-look operation after 6 to 8 weeks. In a series of 10 patients with pan involvement, Sugarman and Kiely[341] reported 8 infants surviving to undergo a second procedure. Resection of necrotic segments plus anastomosis was performed successfully, but the long-term survival rate was only 50%.

### Stoma Closure and Complications

There is neither an ideal weight and age nor a universally agreed-upon time at which intestinal continuity should be restored. The principal determinants are time since surgery, weight gain, and stoma output and need or effects of TPN on overall metabolic function (i.e., liver). In general, the enterostomy may be safely closed anytime after 4 weeks since the last operation. Attempted closure at less than 4 weeks postoperatively may be met with a peritoneal cavity that is obliterated by vascular adhesions and resolving inflammation. Before enterostomy closure, patency of the distal end of the bowel (i.e., colon) should be confirmed by either a retrograde or antegrade contrast study to rule out a stricture or strictures in the distal defunctionalized bowel. A study by Musemeche[342] examined the complication rate after stomas were closed less than 3 months after surgery, 3 to 5 months after surgery, and more than 5 months after surgery; no differences were found. They also noted no difference in complications between patients who underwent closure at a body weight less than 2.5 kg, 2.5 to 5 kg, or greater than 5 kg.

Although enterostomy in neonates may be lifesaving, it is also a major cause of morbidity. In recent studies, enterostomies in newborns had an associated complication rate ranging from 34% to 68%.[343,344] Complications included wound infections, wound dehiscence, stoma stenosis requiring revision, incisional hernia, parastomal hernia, prolapse or intussusception, and small bowel obstruction.

# Survival

Over the past decades, the survival of infants with NEC has progressively improved. This improvement has been attributed to earlier diagnosis and more effective supportive treatment for premature infants. Effective supportive treatment includes improved ventilatory strategies, surfactant therapy, total parenteral nutrition, improved understanding of the pathophysiology and management of critically ill newborns,

and advancements in pediatric anesthesia. The increased survival has been most noticeable in infants who weigh less than 1000 g and are less than 28 weeks' gestational age. In a recent review of 754 premature infants born between 22 and 25 weeks' gestation, the overall survival rate was 63%, with a range of 14% at 22 weeks' gestation to 76% at 25 weeks' gestation.[19] Mortality was still significantly higher in VLBW infants than in larger patients. This is highlighted by studies that examined the outcome of VLBW infants in comparison with "standard" premature infants (>1000 g) with pan involvement NEC. Snyder[345] found that infants weighing less than 1000 g are more likely to require laparotomy (51% vs. 34%) and to eventually have pan involvement (10% vs. 4%) than infants greater than 1000 g. Pan involvement was associated with 100% mortality in both groups. In a retrospective study of 70 infants weighing less than 1000 g with perforated NEC, Erhlich[331] demonstrated that infant survival was independent of the type of surgical treatment (PD vs. laparotomy), but instead was inversely related to the number of comorbid conditions associated with the patient.

Taken together, it seems unlikely that the significant differences in mortality rates observed in various series are attributable to differences in the effectiveness of the treatment programs used. In different groups of patients, the disease varies from predominantly localized disease to extensive necrosis. The patient population differs between the various series. The mortality rate can vary considerably, depending on birth weight, coexisting disease, virulence of the disease process, and whether the patient is inborn locally versus transferred from another facility that has initiated therapy. The precarious state of patients at risk for NEC is emphasized by one series that compared patients who had NEC with matched controls, in which the mortality rate in controls was 33%.[346]

# Complications

## GASTROINTESTINAL

### Intestinal Strictures

The first clinical and radiologic description of intestinal stricture after recovery from acute NEC was reported in 1968 by Rabinowitz.[347] The reported overall incidence varies from 9% to 36%,[348,349] and stricture formation is more frequent after nonoperative treatment. The incidence of strictures after NEC is increasing as the mortality rate from the disease decreases. Strictures result from fibrotic healing of an area of severe ischemic injury. Regardless of whether the stricture follows operative or nonoperative therapy, the most common site of involvement has been the colon (80%). The next most common site is the terminal ileum (15%). Sixty percent of colonic strictures involve the left colon, and the most common colonic site is the splenic flexure (21%). Most patients have single strictures, but multiple strictures can occur (15%).[350] An intestinal stricture should be suspected after nonoperative management of NEC in an infant with failure to thrive, rectal bleeding, or bowel obstruction. These signs and symptoms occur in 50% of patients with strictures and should be evaluated with a contrast enema. If the study demonstrates a stricture in a symptomatic patient, elective resection with anastomosis is usually indicated.

### Intestinal Malabsorption and Short-Bowel Syndrome

Malabsorption may result from a variety of factors including decreased bowel length, decreased mucosal absorptive area, enzyme depletion, gut hypermotility, hypersecretion of gastric acid, bacterial overgrowth, decreased intestinal transit time, vitamin $B_{12}$ deficiency, and bile salt deficiency. Short-bowel syndrome (see Chapter 86) is the most serious long-term GI complication associated with surgically treated NEC. It occurs in up to 23% of NEC survivors who undergo surgical resection.[313]

### Cholestatic Liver Disease

Cholestatic liver disease results from a number of factors but primarily from prolonged administration of total parenteral nutrition. It is characterized by direct hyperbilirubinemia, hepatomegaly, and elevated aminotransferase levels. Although the condition is multifactorial, the most important contributing factor is probably prolonged fasting. It has been shown that the most effective treatment is establishment of early, small-volume enteral feeding that aids in bowel adaptation by conferring a trophic effect on the intestinal mucosa and by stimulating bile flow.

### Recurrent Necrotizing Enterocolitis

NEC can recur after operative and nonoperative management. The incidence of recurrence has been reported to be 4% to 6%.[309,351] No consistent association has been noted between recurrent NEC and the type or timing of enteral feeding, the anatomic site, or the method of initial management. Various case reports suggest supraventricular tachycardia, percutaneous transluminal angioplasty, and allergic enterocolitis to be associated with recurrent necrotizing enterocolitis.[352–354] Interestingly, Pickard[355] proposes that infants with congenital heart diseases and NEC have a decreased risk of having recurrent NEC (OR for CHD 0.58 [95% CI, 0.18 to 1.89], OR for PDA 0.49 [95% CI, 0.10 to 2.28], OR for all other congenital cardiac diseases 0.72 [95% CI, 0.15 to 3.34]) through a retrospective study of 202 infants with NEC. More than 70% of patients were successfully treated nonoperatively for recurrence by Stringer and colleagues.[351]

### Anastomotic Ulceration

A late complication that may occur many years after resection for NEC is the development of anastomotic ulceration. Sondheimer[356] reported six children who underwent ileocolonic resection and anastomosis in the neonatal period in whom lower GI bleeding developed at 5 to 13 years of age. Anastomotic ulceration was diagnosed by colonoscopy, and treatment entailed ulcer resection in five of six patients. Recurrence of marginal ulcers developed in four of five patients who underwent resection. Histologic examination revealed shallow ulcers penetrating only to the muscularis. The cause of the ulcers is unknown.

### NEURODEVELOPMENTAL COMPLICATIONS

The length of hospitalization of infants has been strongly associated with developmental progress at 1 to 2 years of age.[349] This probably reflects the adverse effects of medical and social factors on the developing brain. It is recommended that developmental screening be performed every 4 months during the first year and every 6 months during the second year of life as long-term follow-up data suggest that normal premature infants and survivors of severe NEC remain at high risk for neurologic developmental morbidity.

Approximately 50% of infants surviving NEC are neurodevelopmentally normal.[309,346] Historically, it was believed that any adverse neurodevelopmental outcome in a patient treated for NEC was due to underlying prematurity and comorbid conditions rather than NEC itself, but recent evaluations of surviving infants contest this assumption. Vohr[357] studied the neurodevelopmental, neurosensory, and functional outcomes of 1151 ELBW (401 to 1000 g) survivors at 18 to 22 months' corrected age and reported significant deficits in neurologic development (25%), a Bayley II Mental Developmental Index less than 70 (37%), a Psychomotor Development Index less than 70 (29%), vision impairment (9%), and hearing impairment (11%). NEC was specifically associated as a risk factor for both an abnormal neurologic examination and a low Bayley Psychomotor Development Index. In a study assessing the effect of NEC on neurodevelopment, Sonntag[358] compared VLBW infants with NEC with matched infants without NEC at 12 and 20 months' corrected age. Despite normal somatic growth in infants with NEC not complicated by short bowel syndrome, the authors demonstrated significant neurodevelopmental delay at both 12 and 20 months of age. Fifty-five percent of infants with NEC were noted to be severely impaired versus only 22.5% of infants without NEC. Furthermore, Hintz[359,360] demonstrated through the National Institute of Child Health and Human Development Neonatal Research Network Registry that among the ELBW infants (weight < 10th percentile for gestational age), infants with surgical intervention but not medical treatment for NEC are at significant risk for Mental Developmental Index less than 70 (OR: 1.61, 95% CI, 1.05 to 2.50), Psychomotor Developmental Index less than 70 (OR: 1.95, 95% CI, 1.25 to 3.04), and neurodevelopment impairment (OR: 1.78, 95% CI, 1.17 to 2.73) compared with infants without NEC.

## Prevention

Attempts to reduce the incidence of or prevent NEC must consider the probable pathogenesis of the disease and some of the putative perinatal risk factors. Investigations into preventive measures for NEC including limiting the nosocomial spread of microorganisms, augmenting host defense, decreasing bacterial colonization and overgrowth in the GI tract, and providing factors that enhance intestinal maturation and attenuate the inflammatory cascade may be useful. Infection control measures, breast-feeding, cautious feeding of sick premature babies, immunoglobulin supplementation of feedings, corticosteroid therapy, administration of growth factors, and the use of inflammatory mediator antagonists are some of the preventive strategies that have been studied.

### INFECTION CONTROL MEASURES

Adoption of infection control measures in the nursery may limit the incidence and restrict the spread of infections, thereby potentially eliminating the epidemic waves of NEC.

It has been demonstrated that the initiation of infection control measures stops epidemics of NEC.[219,361] During clustered occurrences, identical microorganisms can be isolated from both the afflicted neonates and their caretakers.

## AUGMENTATION OF HOST DEFENSE

### Oral Immunoglobulin Preparations

The protective immunoglobulins, principally IgA, are deficient in the premature gut. In the absence of breast-feeding, there are only trace amounts of secretory IgA and gut-associated IgG and IgM. Secretory IgA acts by binding bacteria and preventing their attachment to the intestinal mucosa. Eibl[13] demonstrated that enteral administration of an IgG-IgA preparation decreases the incidence of NEC. Their randomized trial involved feeding 179 high-risk infants weighing 800 to 2000 g a human preparation of IgG and IgA with their formula, whereas controls received formula alone. Neither group received breast milk. No cases of NEC developed in the immunoglobulin group, but 6 cases developed in the 91 controls. In a recent study in rabbits, Dickinson[362] demonstrated that IgA supplementation in feedings prevented bacterial translocation by enhancing gut mucosal barrier functions. This effect was not seen with IgG or lactoferrin. In a randomized double-blind controlled trial, enteral IgG supplementation in infants failed to reduce the incidence of NEC.[363] A Cochrane review[364] of three eligible trials (total of 2095 infants) on oral administration of IgG or IgG/IgA combination for preventing NEC did not yield any statistically significant reduction in the incidence of definite NEC (RR 0.84, 95% CI, 0.57 to 1.25), suspected NEC (RR 0.84, 95% CI, 0.49 to 1.46), need for surgery (RR 0.21, 95% CI, 0.02 to 1.75), or death from NEC (RR 1.10, 95% CI, 0.47 to 2.59). Similarly, a meta-analysis of three trials using antistaphylococcal immunoglobulins (INH A-21 and Altastaph) indicated no significant differences in the risk of staphylococcal infection and NEC between either of the antistaphylococcal immunoglobulins with placebos.[365]

### Maternal Glucocorticoid Administration

Glucocorticoids have been shown to accelerate epithelial cell maturation and improve gut barrier function including reduced mucosal uptake of macromolecules, decreased colonization with aerobic bacteria, reduced bacterial translocation, and increased activity of enzymes such as lactase, maltase, sucrase, and Na/K-ATPase.[366,367] Glucocorticoids have also been shown to down-regulate the inflammatory response by stimulating the enzymatic degradation of PAF.[165] These observations have been made both experimentally and clinically.[13,14] Bauer[368] retrospectively noted a significant reduction in the incidence of NEC in babies born to mothers who received antenatal glucocorticoids for fetal pulmonary maturation as compared with controls (2% vs. 7%). This large, multicenter, placebo-controlled trial was well controlled for many potentially confounding variables. Halac[14] conducted a randomized controlled trial of prenatal glucocorticoid administration to mothers in preterm labor. The control mothers received placebos, but their infants received an immediate postnatal course of high-dose dexamethasone for 7 days. The rate of NEC within and between groups significantly decreased after prenatal and postnatal steroid treatment. Although postnatal therapy did not decrease the

incidence as effectively as prenatal therapy did, it improved the clinical outcome of NEC; however, many potentially confounding factors were not assessed in this study. Confirmatory prospective data and assessment of potential postnatal toxicity are necessary. In a double-blind randomized controlled trial comparing perinatal morbidities between antenatal betamethasone and dexamethasone (299 women),[369] no significant differences were observed to exist in the incidence of NEC (0 of 181 for betamethasone, 2 of 178 for dexamethasone). Furthermore, another multicenter randomized controlled trial[370] of 502 pregnant women demonstrated no difference in the incidence of NEC between weekly administration and a single course of antenatal corticosteroids (RR 1.06, 95% CI, 0.44 to 2.56, P value = 0.90).

### Breast Milk

Breast milk decreases the risk for a number of neonatal infections including lower respiratory tract illness, otitis media, bacteremia, meningitis, and NEC.[187] Human milk provides an array of humoral and cellular antiinfective factors, growth factors, and probiotics, as well as essential vitamins and nutrients. Milk factors include IgA, macrophages, lymphocytes, components of the complement system, lactoferrin, lysozyme, transferrin, interferon, EGF, alpha fetoprotein, erythropoietin, the probiotics *Bifidobacterium infantis* and *Lactobacillus acidophilus,* PAF acetylhydrolase,[174] and several inflammatory mediators.[188,371] Strong evidence exists for the protective role of secretory IgA, the main immune component of the enteromammary axis. Breast milk also inhibits the growth of *E. coli* by providing an acidic environment, promoting competitive growth of *Lactobacillus bifidus,* and iron binding (an element essential for the growth of *E. coli*).[372]

Because invasion by infectious agents seems to be a prime factor in the pathogenesis of NEC, breast milk appears to be ideally suited to protect the infant against the disease. Administration of breast milk prevents experimental NEC.[372,373] Formula-fed babies have four to six times the incidence of NEC as breast-fed infants do.[374] A meta-analysis done by Quigley of five trials demonstrated a statistically significantly higher incidence of NEC in the formula-fed group versus the donor breast milk group (RR 2.5, 95% CI, 1.2 to 5.1; risk difference 0.03, 95% CI, 0.01 to 0.06; number needed to harm 33, 95% CI, 17 to 100).[375] Subsequently, two more clinical trials were conducted. The analysis of 1272 infants in the National Institute of Child Health and Human Development Neonatal Network Glutamine Trial showed a reduction of the likelihood of NEC or death after 14 days of diagnosis by a factor of 0.83 for every 10% increase in the proportion of total intake of human milk. For every 100 mL/kg increase in human milk intake with the 14 days of diagnosis, there is a decreased risk of NEC or death (hazard ratio 0.87, 95% CI, 0.77 to 0.97).[376] Similarly, Sullivan[377] showed in a trial of 207 infants (500 to 1250 g) comparing an exclusively human milk–based diet with a diet of both human milk and bovine milk–based products that there was a reduction in NEC of 50% and in surgical NEC of almost 90% in infants fed an exclusive human milk diet. The numbers to treat with an exclusively human milk–based diet to prevent 1 case of NEC and 1 case of surgical NEC were 10 and 8, respectively. The last two trials imply a dose effect of human milk in the reduction of both medical and surgical NEC.

## Feeding Practices

There is little disagreement that NEC is more common in fed infants and that bacterial overgrowth is facilitated by the substrate provided by formula. Although the potential benefit of carefully regulated feeding practices to prevent NEC is widely accepted, randomized trials have failed to demonstrate any difference in the incidence of NEC related to fast versus slow, early versus delayed, or continuous versus intermittent bolus feeding.[35–39,378–381]

## METHODS TO DECREASE INTESTINAL BACTERIAL COLONIZATION AND OVERGROWTH

### Administration of Probiotics

Probiotic bacteria are defined as "live microbial supplements that colonize the intestine to provide benefit to the host."[382,383] The use of anaerobic bacterial supplementation in the treatment or prevention of GI disease has been well described. There has been increasing interest in using probiotics for the prevention of NEC. Probiotic microorganisms commonly used are strains of *Lactobacillus, Bifidobacterium, Streptococcus salivarius,* and *Saccharomyces boulardii.* Multiple randomized, controlled trials have attempted to address this issue, and several meta-analyses have been published. The most recent meta-analysis by Deshpande[384] analyzed 11 trials[385–395] ($n = 2176$) in which enteral probiotic supplementation was started within the first 10 days and continued for 7 or more days in VLBW neonates (<1500 g). Compared with the control group of no probiotics, significant reductions of risk were noted for developing definite NEC (RR 0.35, 95% CI, 0.23 to 0.55, $P < 0.00001$) and dying from all causes (RR 0.42, 95% CI, 0.29 to 0.62, $P < 0.00001$). Further sensitivity analysis, examination through the funnel plot, and trial series analysis supported a 30% reduction in the incidence of NEC (alpha = 0.05; power of 80%). However, risks for sepsis, death from NEC, and longer time to full feed (120 to 150 mL/kg per day enteral feeds or as per the prestated definitions by authors) did not differ significantly between both groups after adjustments were made for heterogeneity via a random-effects model.

Rachmilewitz[396] and Mackey[397] considered applying similar concepts used in probiotic feedings by treating infants with isolated microbe-associated molecular patterns (MAMPs) to induce protective responses that promote intestinal health. An MAMP is a molecular sequence or structure in any pathogen-derived molecule that directly interacts with TLRs on the intestinal epithelium. It has been shown to effectively improve symptoms of colitis in mice.[396] The idea of administering inactivated probiotics (heat-killed commensals) or bioavailable TLR ligands that can potentially induce beneficial TLR-mediated protective effects without the risk of infection from administering probiotics is being actively investigated.

### Administration of Prebiotics

Besides probiotics, other researchers have suggested administering prebiotics, which are nondigestible dietary supplements such as long chain carbohydrates or mucins that promote proliferation of beneficial commensal bacteria. A meta-analysis of four trials ($n = 126$) by Srinivasjois[398] examined the efficacy and safety of prebiotic oligosaccharide supplementation of formula in reducing the incidence of NEC and sepsis. The duration of the supplementation ranged from 14 to 33 days. The analysis indicated no NEC in one trial, but the rest did not report specifically on NEC or sepsis. Two of the four trials demonstrated a significant increase in bifidobacterial counts in the prebiotic supplemented group. The major advantage of prebiotic supplements is the lack of live microorganisms, which reduces the risk of infection that may exist for the use of probiotics.

### Administration of Postbiotics

Similar to prebiotics, postbiotics, which are bacterial metabolites, may also be a potential treatment for NEC by generating some beneficial effects on the intestinal flora. Butyric acid, a short-chain fatty acid produced by commensal bacteria in the colon through anaerobic catabolism of complex carbohydrates, is a major energy source for colonic enterocytes and has a widely recognized but poorly understood role in intestinal growth and differentiation,[399,400] inflammatory suppression,[401] and apoptosis.[402] It has been administered with limited success in human inflammatory bowel disease.[403]

### Enteral Antibiotics

Nonabsorbable broad-spectrum antibiotics that inhibit bacterial growth have been administered in an effort to prevent NEC. The use of an enteral aminoglycoside (e.g., kanamycin or gentamicin) has been proposed as a means to decrease the incidence of perforation during nonoperative treatment. However, randomized, controlled studies found no difference in the clinical course, complications, or mortality rate between infants who received the antibiotic and those who did not.[404,405] In addition, oral aminoglycosides may be absorbed across the damaged gut, which can lead to increased serum levels and thereby potentially contribute to drug toxicity. Resistant strains of bacteria emerged after treatment with enteral kanamycin,[404] and there is the omnipresent risk for promotion of the growth of fungal species. In a recent randomized placebo-controlled study of oral vancomycin in preventing NEC in preterm infants, Siu[16] reported a 50% reduction in the incidence of NEC in the vancomycin group in comparison with controls. A meta-analysis of five trials ($n = 456$) by Bury[406] suggested that prophylactic enteral antibiotics resulted in a statistically significant reduction in NEC (RR 0.47 [0.28, 0.78]; RD −0.10 [−0.16, −0.04]; NNT 10 [6, 25]) and in NEC-related deaths (RR 0.32 [0.10, 0.96]; RD −0.07 [−0.13, 0.01]; NNT 14 [8, 100]). However, the summary analysis of three trials gave an increase in the incidence of colonization with resistant bacteria that was just significant (RR 1.73 [1.00, 2.97]; RD 0.07 [0.00, 0.13]). Furthermore, a subsequent retrospective cohort analysis of 5693 neonates (<1000 g) from the NICHD database by Cotton[407] demonstrated that for every antibiotic treatment day, there was at least a 4% increase in the odds of an infant having NEC or dying, especially when antibiotics were initiated in the first 3 postnatal days for 5 or more days of treatment.

The available studies do not support the routine administration of enteral antibiotics to all high-risk premature infants, many of whom have poor intestinal motility. Effectiveness has not been proved, and resistant organisms may develop.

Administering specific antibiotics to infants may be indicated in nurseries in which an outbreak of NEC is associated with a specific organism.

## METHODS TO DETER THE INFLAMMATORY CASCADE

### Inflammatory Mediator Antagonists

The effects of PAF are mediated by receptors, and many compounds that function as receptor antagonists or enzymes that degrade these proteins have been described. Animal experiments using PAF antagonists or PAF-degrading enzyme (PAF acetylhydrolase) have demonstrated the capacity to prevent bowel injury produced by the administration of endotoxin or hypoxia in rats.[47,115] PAF acetylhydrolase is also known to be present in breast milk. Despite promising results in animal models, no human trials using PAF antagonists or degrading enzymes in the treatment of NEC have been reported.

### Arginine

While NO has been linked to the pathogenesis of NEC, it may be beneficial to neonate intestine by regulating mucosal blood flow, inflammatory signaling, barrier function, and wound healing.[408] Arginine, a substrate for the production of NO, has been shown to be in low level in neonates with NEC as well.[153,409] Thus researchers tested the effect of arginine supplementation on the incidence of NEC. In the 2007 Cochrane Review[155] one trial[154] on arginine supplementation was reported to reduce the incidence of all stages of NEC (RR 0.24, 95% CI, 0.10 to 0.61; RD -0.21, 95% CI, -0.32 to -0.09), but further studies are necessary to confirm and elucidate the mechanism of this benefit.

### Epidermal Growth Factor

As discussed in previous sections, EGF is a growth factor that exerts its effects by binding to the EGF receptor. It has multiple effects including healing of damaged mucosa by inducing mucosal enzyme and trefoil peptide expression and inhibiting effects on gastric acid secretion.[62,65–67] Clark demonstrated intestinal protection by accelerating goblet cell maturation and mucin production and normalizing expression of tight junction proteins in a neonatal rat model. One prospective randomized controlled study[410] of eight neonates with NEC showed measurable trophic effects on the gastrointestinal mucosa and no significant difference in clinical safety between a 6-day continuous intravenous infusion of EGF and placebo. The administration of EGF is still at the testing stage. Further studies are necessary before any clinical use.

*The complete reference list is available online at www. expertconsult.com.*