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Pathophysiology 21 (2014) 29–34

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Feeding associated neonatal necrotizing enterocolitis (Primary NEC) is an inflammatory bowel disease

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Abstract

Neonatal necrotizing enterocolitis which develops after feeding preterm infants is characterized by severe intestinal inflammation and profound systemic metabolic acidosis. The fermentation of undigested dietary carbohydrate by colonic flora yields gases (CO_2 and H_2) and short chain organic acids. These organic acids can disrupt the intestinal mucosa and initiate inflammation driven predominantly by resident mast cells and by granulocytes which are recruited from blood. A systemic acidosis ensues derived from intestinal acids, not classic lactic acidosis produced from anaerobic metabolism. The systemic acidosis further compromises inflamed bowel leading to bowel necrosis.

Keywords: Necrotizing enterocolitis; Inflammatory bowel disease; Organic acids; Mast cells

Neonatal necrotizing enterocolitis (NEC) is a devastating illness. Well over 20,000 articles from over 10,000 individual authors have been written in the past 30 years (Google Scholar Search – June 2013). The incidence of NEC in the United States is approximately 3–10% of pre-term very low birth weight babies (birth weight <1500 g), which translates into at least 2500 cases of NEC annually [1–4]. It is one of the most common causes of death in premature babies surviving respiratory distress.

Signs and symptoms associated with NEC are noted in Table 1. There are radiographic diagnostic criteria for more advanced disease [4–6]. Pneumatosis intestinalis, with or without hepatic portal venous gas, is considered as pathognomonic of NEC. Free peritoneal air resulting from bowel wall perforation or a sentinel loop due to extensive bowel wall necrosis indicates an advanced stage of NEC. Laboratory investigations reveal elevation of acute phase reactants similar to neonatal sepsis syndrome, thrombocytopenia and metabolic acidosis, all in the context of inflamed and necrotic intestine from a disrupted intestinal mucosa [7–10].

NEC is not a specific diagnosis but a constellation of signs and symptoms with several proposed etiologies. Spontaneous intestinal perforation (SIP) presenting as pneumoperitoneum has been recognized as a distinct entity from NEC. SIP often presents early without pneumatosis or portal venous gas [11]. In a sick preterm infant the distinction may not be resolved until surgical resection or autopsy.

For the purpose of this review we will broadly classify NEC into two main categories: (1) Primary NEC which typically occurs in an apparently stable preterm infant who is feeding enterally with no recognizable prior triggering event and (2) Secondary NEC which afflicts a preterm or term infant, who may or may not be feeding. These babies almost always have a recognizable triggering condition [7,8].

Primary NEC (lack of any inciting trigger) occurs beyond the first week of postnatal life and accounts for the majority of NEC (85–90% of cases) in neonatal intensive care units [2]. Secondary NEC, a subset of which has been termed spontaneous intestinal perforation (SIP) comprises about 10–15% of all cases each preceded by a proximate cause [2–4]. Examples of Secondary NEC (SIP) include term infants with congenital cyanotic heart disease, polycythemia, post-exchange transfusion, hypoxemic-ischemic insult with multiple organ failure and preterm infants with transfusion associated NEC [2,4]. There is no national database for reporting the various forms of NEC. Once NEC is clinically apparent, the clinical features of both categories look similar with varying degrees of severity.

In Secondary NEC, earlier recognition and avoidance of inciting events as well as careful monitoring of the infants who have them may either prevent NEC or result in

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http://dx.doi.org/10.1016/j.pathophys.2013.11.006

Table 1
Signs of necrotizing enterocolitis.

Systemic	Gastrointestinal
Temperature instability	Abdominal distension
Lethargy	Abdominal wall discoloration
Apnea/bradycardia	Fewer stools with increased feeds
Hypotension-shock	Feeding intolerance (residuals)
Metabolic acidosis-mixed	Emesis (bilious)
Glucose instability	Carbohydrate intolerance
Left-shifted WBC differential	Positive stool clinitest
Neutropenia	Breath hydrogen elevated
Thrombocytopenia	Bloody stool
Eosinophilia	Gross blood 25%
Coagulopathy	Occult blood 25–60%
Positive blood culture	
Elevated C-reactive protein	

early detection and prompt management of NEC, potentially improving clinical outcomes. There is no such strategy applicable to Primary NEC since it sets in without warning events in an otherwise stable preterm infant who is either on full or substantial amount of enteral feeding. Many factors such as osmolality of formula, timing and advancement of feedings, and primary gastrointestinal infection have been proposed as the inciting event but none have proven significant. Exclusive breast feeding has been shown to lower the incidence [9,10].

There are several excellent articles describing potential and detailed subsets of pathophysiology that should be considered [4,11]. This manuscript focuses on the broad view of the initiation and propagation of Primary NEC. NEC is the end result of a process and is not caused by the same pathophysiology in every premature infant. Vascular (ischemia reperfusion) injury and infection are infrequent causes of NEC and both are considered as Secondary NEC. Primary NEC is an inflammatory process without a clear understanding of its genesis. Both lead to intestinal necrosis but there remain specific subtleties differentiating them [3,6]. Without regard to the cause of severely damaged intestine, the treatment is the same, including cessation of feedings, gastric decompression, parenteral nutrition, supportive care, correction of acidosis, hypotension and blood loss, broad-spectrum antibiotics, and transfusion as necessary. Serial examinations are performed and abdominal X-rays are taken in an attempt to detect intestinal perforation early. A perforation may be managed by peritoneal drainage or a laparotomy to resect necrotic bowel, commonly leaving a diverting intestinal stoma. Unfortunate complications include short bowel syndrome, strictures, adhesions, entero-colonic fistulas, peritoneal abscesses and various complications of prolonged parenteral nutrition.

1. What is known about NEC

The intestine of the low birth weight premature infant is immature in many of its functional aspects [13]. There is decreased digestion and absorption of many essential nutrients. There is limited ability to both secrete enzymes as well as decreased intestinal motility. Undigested carbohydrate is substrate for intestinal flora which produce organic acids and gases. The liver which metabolizes a number of compounds absorbed from the intestine does so with variable degrees of success. The preterm infant's immature intestinal barrier is less efficient at controlling toxins and organisms, specifically bacteria and viruses that are able to induce mucosal disruption. These organisms may initiate a local inflammatory response releasing mediators that are toxins. These should be removed by the liver, to prevent a severe systemic response. Table 2 delineates the characteristics of Primary and Secondary NEC [12,14,15]. The unfed infant commonly has multiple organ involvement before the bowel damage which is limited to a single site, commonly within the small bowel [11]. If the infant has been fed, the onset is much later (beyond 7 days) with over 25% of cases occurring after a month of age. The affected site is the distal ileum and proximal colon where undigested substrate (carbohydrate) first meets a significant load of bacteria. The intestine is the first organ affected and then there is subsequent systemic illness. A key difference between the two (Table 2) is that the acidosis of Secondary NEC is lactic acidosis whereas the acidosis of the late onset form (Primary NEC) is a mixed metabolic acidosis comprising more than one short chain fatty acid or organic acid [6,12].

Clinical studies are hampered, because many key data elements are not considered or reported, including the prenatal history, time of rupture of the membranes, prenatal steroids or antibiotics, the mode of delivery, post natal steroids, the management of respiratory distress, the microbiology of the individual baby as well as of the environment of the NICU. Most studies are unfunded and are commonly retrospective as a secondary or tertiary analysis. Many important variables are missing, including events preceding the onset of NEC in the individual baby. Because there has been no well-defined etiology, investigator bias adds to the confusion. In general, the prevailing basic theory is that the preterm infant is born with multiple limitations of an immature intestine including malabsorption, immune dysregulation, a poorly regulated intestinal circulatory system, poor intestinal motility, and mucosal barrier defects [3,4,11,14].

Animal models of NEC have had limited utility. There is no premature primate NEC model. The rat and mouse models of NEC have been called into question, given that they depend on a combination of significant hypoxia combined with cold stress, neither which are likely to be sustained insults in the appropriate care of the premature baby.

Associations that are critical include prematurity with an inverse relationship, increasing incidence of NEC as gestational age decreases [1,2,4,6,14]. The feeding association is important as carbohydrate metabolized by the bacteria is the source of organic acids in the intestinal lumen, and the gases of pneumatosis intestinalis and portal venous tree. Timing is important because subclinical cases may recover within several days once cautious feeding and supportive care have

Table 2
NEC – Primary and Secondary.

	Primary (fed infant)	Secondary (unfed infant)
Gestational age	<34 weeks, <1500 g	Preterm to term
Perinatal asphyxia	Unlikely	Common
Bacterial colonization	Well established after oral feedings	Light, little oral nutrition
Onset of NEC	7 days to 3 months (25% >1 month)	<7 days after birth
Location of necrosis	Ileum, colon	Small bowel
	Multiple sites common	Single site
	Pneumatosis intestinalis	
	Portal venous gas	
Organs systems involved	Intestine first, then systemic	Multiple (brain, kidneys, heart, liver)
Acidosis	Mixed metabolic	Lactic acid
	Propionic, butyric	
Blood culture	Positive ∼25%	Sterile
	Commonly Gram negative bacteria	

been initiated. A concept which is no longer relevant is the diving seal reflex. It is a mature reflex not found in seal pups. Osmolarity is not an issue since the gastrointestinal tract is designed to dilute and concentrate nutrients. The stomach which by initial exposure is the most susceptible organ to hyperosmolarity is rarely involved.

The classic theory does not correlate well with the epidemiology. About 90% of patients afflicted with NEC are preterm, less than 34 weeks gestation, and have been enterally fed, many for several weeks [3,12,14]. Most of the babies have gained weight demonstrating maturing intestinal function which then becomes impaired. The majority of the babies have not had an apparent hypoxic-ischemic insult and one quarter of the cases of NEC occur beyond a month of age, suggesting perinatal events have little to no significance in Primary NEC. Therefore, the etiology of NEC falls into two broad categories; Secondary NEC encompassing infection, approximately 5%, and ischemia reperfusion injury 5–10%. Primary NEC describes a neonatal form of inflammatory bowel disease which accounts for 85–90% of all the clinical cases of NEC.

Ischemia-reperfusion injury has been reported. However, the majority of the babies that have ischemic risk factors do not develop NEC. Most babies who develop Primary NEC have no identifiable ischemic insult [15]. There are also many babies who do not develop NEC even though they have obvious, severe ischemic insult. There is no correlation with NEC of premature babies and acute tubular necrosis, perinatal asphyxia, and intraventricular hemorrhage. Animal models of ischemia have been the Primary NEC model since the 1960s. In a sheep model, event reducing the hematocrit to 30% and giving a hypoxic insult of 10% oxygen for 30 min, oxygen consumption was not compromised as there was increased tissue O_2 extraction and therefore no intestinal damage [16]. In a canine model of NEC, two hours of hypoxia and resuscitation, if the dogs were sacrificed within 24 h there was intestinal vascular congestion and hemorrhage [17]. If the animals were allowed three days before sacrifice there was full recovery of the intestine with normal histology. The intestine has the ability recover if there is not a continued insult.

Regarding infection, there are many reported cases of neonatal appendicitis, Hirschsprung's disease, colonic obstruction, diverticulitis and other forms of localized inflammation that have some of the characteristics of necrotizing enterocolitis but with a clear underling etiology. In each of these examples enteric bacteria play a primary role [18,19].

At birth newborns are sterile but there is rapid colonization of skin, umbilical cord, oropharynx, and intestinal tract. Once the intestine is colonized with as many as 500 million to 1 billion bacteria/gram of stool, portal bacteremia is relatively common [18]. An efficient liver prevents that threat from becoming systemic.

A number of organisms have been reported as associated with necrotizing enterocolitis [18]. These include viruses: coronavirus, rotavirus, enterovirus, and parvovirus B19 [20,21]. The viruses cause disease by direct infection. The associations with bacteria have been more spotty and include, *E. coli*, *Klebsiella* species, *Enterobacter* species, and *Staphylococcus* species. Bacteria have several means by which to damage the intestine, including toxin production [19]. Bacteria also ferment carbohydrates of any variety with lactose being the most commonly malabsorbed carbohydrate in milk (breast milk or formula). The fermentation process of bacteria produces several gases, carbon dioxide and hydrogen. Carbon dioxide is soluble and rapidly dissolved. Hydrogen is the gas in the bowel wall of pneumatosis intestinalis as well as the portal gas A series of organic acids (short chain fatty acids) are produced in the fermentation process including lactic acid, butyric acid, propionic acid, acetic acid, isobutyric acid, and formic acid [18]. The speed of fermentation is important. Generating acid quickly initiates local intestinal inflammation and skip lesions. The acids generated are the source of the systemic mixed metabolic acidosis.

The most commonly used medications in the neonatal intensive care unit are ampicillin and gentamicin [22,23]. Carbonero et al. reported that *Klebsiella* species and other Gram negative rods exposed to low dose ampicillin increase their genomic β-lactamase becoming more resistant as well as simultaneously increasing the beta-galactosidase activity

[24]. Therefore, as antibiotic resistances increases carbohydrate fermentation also increases rapidly. This sets the stage for high concentrations of multiple organic acids within the intestine that cause mucosal inflammation, leading to systemic acidosis. The concept of intraluminal organic acids inducing colitis is well described in that organic acids, acetic, butyric acid and propionic acid have been used in animal models to induce inflammatory colonic disease [6,24–27].

Modeled after the concept that *Helicobacter* sp. causes gastric ulcers, recently there has been a concerted effort to determine a specific pathogen responsible for causing the intestinal inflammation of NEC. Several studies have shown there is a change in the intestinal flora in children who develop necrotizing enterocolitis in that the proteobacteria constitute as much as 70% of the organisms in the stool, in cases within three days preceding the clinical diagnosis of NEC [28]. Included in this group are the previously mentioned Gram negative rods *E. coli*, *Klebsialla* sp. and others. Despite the systematic search, no consistent pathogen has been identified by culture techniques or by genomic analysis. The organism colonizes the GI tract while the baby is being fed sterile liquid formula or breast milk. The hypothetical organism would have to be one that preferentially affects the distal ileum and proximal colon, the primary sites of disease in preterm babies. No other intestinal or colonic pathogen has shown any similar pattern. There is no seasonal pattern as is commonly found with intestinal infections. Toxins have rarely been identified in preterm NEC. Although an important etiology for GI disease in older children they are not an issue in newborns in that toxin receptors are not yet developed.

The common intestinal flora found in all babies and in all intensive care nurseries may be the culprits in a subtle way, not by their invasive characteristics nor their ability to produce toxins, but simply by their ability to ferment carbohydrate and produce organic acids more quickly than the capacity of the preterm infants' defense mechanisms, the mucosa, and the portal system and liver can accommodate.

2. Proposed pathogenesis of Primary NEC

To initiate intestinal damage a mechanism to disrupt the mucosal barrier is a central concept. Only three of the many mechanisms of disruption of the intestinal mucosal barrier in animal models [29,30] seem to be of any significance. These are ischemia reperfusion injury, bacterial toxins, and organic acids. Since there is minimal likelihood of bacterial toxins or ischemic reperfusion injury, the organic acids should be the focus. Inflammation likely begins with carbohydrate (lactose) malabsorption and leads to bacterial fermentation and the generation of multiple organic acids, short chain fatty acids which disrupt the mucosal barrier. A local activation of the proinflammatory defense response along with poor motility and stasis initiates intestinal inflammation and potentially necrosis. A number of cells are responsible for the inflammation including cells which initiate and exacerbate the process.

Table 3
Cells inducing intestinal injury.

Initiation	Exacerbation
Enterocytes	Neutrophils-recruited
Mast cells	Eosinophils-recruited
Neutrophils-resident	Monocytes
Macrophages	Lymphocytes
Endothelium	Platelets

These are listed in Table 3. Of particular note here is the mast cell [30–34]. The mast cell has not been well studied since it is consumed in the inflammatory process and is no longer present in necrotic tissue. The mast cell has over 30 different mediators, some rapidly eluted, some from preformed granules, and others secondary or newly generated. Mediators reported associated with NEC (Table 4) include Leukotrienes C4, D4, Interleukin-6,8 oxygen radicals, platelet activating factor and thromboxane all of which can be found in an activated mast cell [33–36].

Of the original list of systemic and gastrointestinal signs and symptoms of feeding-associated Primary NEC the one critical factor is mixed metabolic acidosis. With hypoxic ischemic injury the lactic acidosis is transient and readily managed. The mixed metabolic acidosis of NEC is sustained which often takes many hours to fully resolve. Using tandem mass spectroscopy, we have examined the whole blood organic acid profile of babies in the NICU with severe metabolic acidosis (Fig. 1). Babies ($N = 28$) with hypoxic ischemia injury, and conditions other than NEC have lactic acid as the primary circulating metabolite causing the acidosis. Babies with NEC ($N = 8$) and severe metabolic acidosis

Table 4
Mediators derived from mast cells.

Preformed and rapidly eluted mediators
- Histamine
- Eosinophil chemotactic factors of anaphylaxis (ECF-A)
- Intermediate weight ECF-A
- Neutrophil chemotactic factors
- Superoxide anions
- Exoglycosidases – hexosaminidase, glucuronidase, D-galactosidase
- Serotonin
- Kininogenase
- Arylsulfatase A

Preformed granule-associated mediators
- Heparin
- Chymotrypsin/trypsin
- Peroxidase
- Superoxide dismutase
- Arylsulfatase B
- Inflammatory factors of anaphylaxis

Secondary or newly generated mediators
- Nitric oxide
- Slow-reacting substances of anaphylaxis (leukotrienes C, D, E)
- Prostaglandins
- Monohydroxyeicosatetraenoic acids
- Hydroperoxyeicosatetraenoic acids
- Hydroxypheptadecatrienoic acid
- Thromboxanes
- Platelet-activating factor

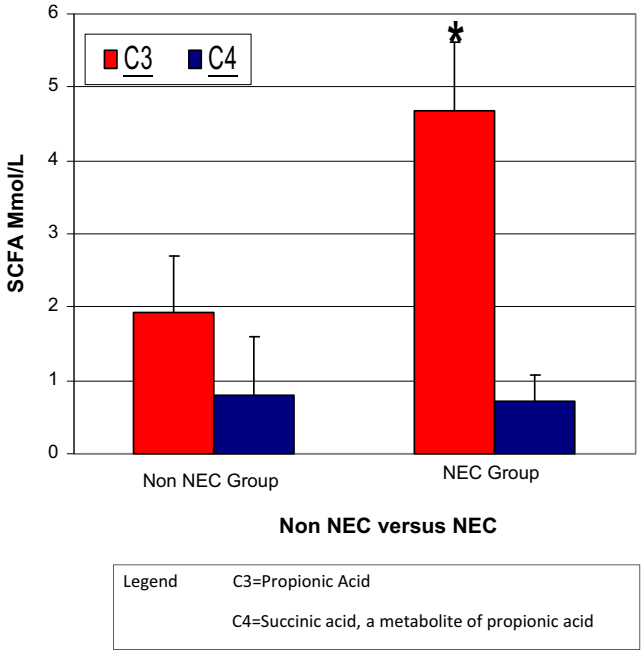

Fig. 1. The circulating organic acids in neonates with systemic metabolic acidosis - Non-NEC versus NEC.

had propionic acid as the primary organic acid. Propionic acid is one of the important metabolic fermentation products of members of the proteobacteria specifically *E. coli*, and *Klebsiella* sp. [24,25]. Endogenous sources of propionic acid result from catabolism of a small number group of amino acids. Protein catabolism is unlikely in preterm infants who are growing after having been fed successfully. The primary source of the propionic acid producing metabolic acidosis is the intestinal bacteria which continue to generate acid as long as substrate is available. This explains the location of NEC as distal and proximal colon where the most rapid fermentation occurs. Fermentation more distal is less likely since the substrate is consumed especially in the context of poor motility, inflamed distal ileum and proximal colon. Based on newborn screening program data from New York State (NY State DOH – personal communication) preterm infants <34 weeks gestation often have limited capacity to clear propionic acid. This organic acid is metabolized only by the liver. Other organic acids such as lactic and short chain fatty acidic with fewer than 4 carbons (C4) are metabolized by the Krebs Cycle present in the majority of tissues. In newborn screening programs propionic acid is a common false/positive in babies who are fed. The propionic acid is elevated prior to 34 weeks gestation and resolves without true disease. This suggests a maturational defect of the liver to metabolize propionic acid. The enzyme (propionyl CoA carboxylase) converts propionic acid to methylmalonyl-CoA and a Vitamin B12 dependent enzyme rearranges it to succinyl CoA the precursor of succinic acid, which is within the Krebs cycle. Prior to birth without intestinal colonization there is no generation of organic acids from the intestine and therefore no need to have the hepatic enzymes functional.

Based on these observations we have developed a piglet intraluminal model of NEC which includes all of the key features, skip lesions, pneumatosis, portal gas, systemic acidosis, thrombocytopenia, neutropenia and others [37]. The model is induced only by a rapid lactose fermenting Gram negative rod incubated with preterm formula. There is no hypoxic or ischemic insult [37]. This model may explain the utility of probiotic administration to reduce the risk of NEC [38–40].

In summary, the initiation of NEC is linked to rapidly fermenting bacteria producing a series of organic acids that cause local intestinal inflammation. Intact sustained blood flow delivers acids along with hydrogen (portal gas) by the portal system to the liver. The liver metabolizes most organic acids efficiently in the term infant but in the preterm baby the liver is overwhelmed leading to systemic acidosis specifically propionic acidemia. The systemic mixed metabolic acidosis, led by propionic acid, exacerbates the damage to already inflamed intestine resulting in intestinal damage (Primary NEC).

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