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The epidemiology and pathogenesis of necrotizing enterocolitis

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Necrotizing enterocolitis (NEC), a syndrome characterized by crepitant necrosis of the bowel, has emerged as the most common neonatal gastrointestinal emergency in many countries of the world. In the United States, NEC strikes 1 to 8% of patients admitted to neonatal intensive care units, almost all of whom are premature infants. The incidence is low in certain countries with a low premature birth rate, e.g., Japan. Two theories of pathogenesis are: the Santulli theory, which implicates three factors: ischaemia, bacteria and substrate; and the Lawrence theory, which stresses the injurious role of bacterial toxins on the immature gut of the preterm infant. Clinical and experimental evidence support each of the theories, but neither theory can explain certain clinical phenomena, particularly the resistance to NEC manifested by more than 90% of preterm infants, who never develop the syndrome. A unifying hypothesis of pathogenesis and a mathematical model of NEC are outlined. Because clustering of cases may occur, the design of clinical trials of preventive measures for NEC must include simultaneous control infants.

Necrotizing enterocolitis (NEC) qualifies as one of the major unsolved problems of perinatal care. Its pathogenesis, and more important, its prevention, remain unsolved after nearly 25 years. Theories and anecdotes abound; well designed epidemiological reports are too few. In this chapter the epidemiology and pathogenesis of NEC are discussed with emphasis on the theories outlined by Santulli in New York [1] and by Lawrence in Brisbane [2]. Some clinical and experimental evidence in support of each of the theories will be cited which unfortunately fail to explain all the clinical phenomena. Finally, a unifying hypothesis will be presented. This has been revised and updated a number of times [3–5], yet remains imperfect nonetheless.

NEC has emerged in the past 25 years as the most common gastrointestinal emergency in the neonatal intensive care unit (NICU) [6]. It is a syndrome characterized by crepitant necrosis of the gut. Ninety percent of its victims are preterm infants, many of whom would have died in earlier times from respiratory complications. In the United States the incidence ranges from 1–3 cases per 1000 live births and 1–7.7% of NICU admissions. The mortality has been calculated at 13 deaths per 100,000 live births, or 20–40% of cases [7].

Clinically, NEC is characterized by the triad of abdominal distension, gastrointestinal bleeding, and pneumatosis intestinalis, i.e. air within the intestinal wall. In addition, infants with severe NEC may have air within the portal vein or pneumoperitoneum following intestinal perforation. In one study at the University of New Mexico, the radiographic findings were correlated with the outcome of NEC, employing a grading system for pneumatosis intestinalis as mild, moderate or severe (Table 1). The mortality was 18%, 21% and 62%, respectively. The presence of portal venous gas at the time of diagnosis of NEC was associated with a 65% mortality. Infants with a combination of severe pneumatosis and portal venous gas had the worst prognosis: a mortality of 86% [8].
outcome of severe NEC was thus predicted by the initial radiographic findings (Table 1).

### Historical aspects

Case reports of neonatal gastrointestinal perforations by Siebold in 1825 [9] probably represent the first reported instances of NEC. Thelander compiled a review in 1939 of 85 neonates with gastrointestinal perforation unassociated with anatomic obstruction of the bowel, some of whom probably had NEC [10]. Perforation of the intestines, particularly of the colon after exchange transfusion, was recognized in the 1950s and amply documented before the clinical syndrome of NEC was fully described [11–13].

The syndrome was named following the report of Rossier in 1959 describing 15 infants, 14 of whom died, with “ulcerative-necrotic enterocolitis of the premature” [14]. The clinical, radiographic and pathologic entity of NEC was clearly described in the 1960s by Berdon, Mizrahi and associates from Babies Hospital in New York [15, 16], one of the first North American centers with an NICU, where a surgical approach to the disease was formulated by Touloukian and Santulli [17].

Since the 1970s, the proliferation of NICU has been associated with an increasing prevalence of NEC, which is now the most common surgical emergency encountered in newborn infants [18].

### The Santulli theory

Santulli and associates in 1975 attributed NEC to the interaction of three essential components to the development of the disease: injury to the intestinal mucosa, the presence of bacteria, and the availability of a metabolic substrate, i.e., formula feedings, in the gut lumen [1]. These three elements could be documented or inferred in most preterm infants who developed NEC, by the mechanisms outlined below.

### Ischaemia

Intestinal ischaemia is the result of decreased perfusion, by a variety of mechanisms, which include vasoconstriction, hypotension, low flow, or thrombosis. Intestinal injury is widely attributed to “the diving reflex”, a mechanism of selective circulatory ischaemia which occurs in response to asphyxia. Classical studies were performed by Scholander, a Scandinavian physiologist, who measured the cardiovascular reflexes of diving mammals and birds [19]. In his experimental model, the seal was immersed in cool water, with an electromagnetic flow probe attached to its flipper. During the simulated dive, the seal’s normal heart rate of 80–90 beats minute fell to 5–6 beats per minute. There was redistribution of blood flow, virtually all of which went to two organs: the heart and the brain. Peripheral arterial pressure decreased, and peripheral flow virtually ceased. When the seal surfaced, after 6 minutes, flow was restored to all the organs which had been ischaemic, including the gut and the kidneys. Reactive hyperemia occurred. It is postulated that this reflex occurs in the preterm infant whose gut is vulnerable to the ischaemic insult, which initiates NEC. In 1990, Lloyd was the first to link the diving reflex to gastrointestinal perforations in the newborn [20]. He noted that, in

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**Table 1. Radiographic abnormalities and clinical outcome in 147 infants with stage II and stage III necrotizing enterocolitis. (From A. M. Kosloske et al [8] AJR 1988; 151: 771-774, with permission.)**

| Radiographic abnormality                      | No. of infants | No. with gangrene | No. with pan-necrosis | No. of deaths |
|----------------------------------------------|----------------|-------------------|-----------------------|--------------|
| Pneumatosis, mild                            | 51 (35)        | 23 (45)           | 4 (8)                 | 9 (18)       |
| Pneumatosis, moderate                        | 57 (39)        | 32 (56)           | 7 (12)                | 12 (21)      |
| Pneumatosis, severe                          | 32 (22)        | 29 (91)           | 18 (58)               | 20 (62)      |
| Pneumoperitoneum                             | 45 (31)        | 45 (100)          | 15 (33)               | 21 (47)      |
| Portal venous gas                            | 23 (16)        | 23 (100)          | 14 (64)               | 15 (65)      |
| Pneumatosis, severe and portal venous gas    | 14 (10)        | 14 (100)          | 11 (65)               | 12 (66)      |

Numbers in parenthesis are percentages. The total number of infants is greater than 147 because some infants had 2 or 3 radiographic abnormalities. Six infants who survived and one who died had intestinal gangrene but no pneumatosis intestinalis. Pan-necrosis, necrosis involving 75% or more total length of jejunum, ileum and colon.
a large group of infants with perforations of the gastrointestinal tract, 80% had experienced a significant episode of asphyxia or shock during the perinatal period. Amongst preterm infants in the NICU, apnoea, bradycardia and respiratory failure requiring endotracheal intubation are common events.

Prenatal circulatory events may predispose the NEC. Malcolm and associates monitored flow velocity in the umbilical artery of high risk pregnancies, and found a highly significant increase in risk for NEC among fetuses with absent or reversed end diastolic flow velocity, 53% of whom developed NEC, compared to a 6% occurrence among matched controls [21].

Catheterization of the umbilical artery, an invasive yet common procedure, may induce intestinal ischaemia. After advancement of the catheter tip into the abdominal aorta, blanching or cyanosis of an infant’s lower extremities is an occasional observation. After the catheter is removed, the blanching or cyanosis promptly resolves. Similar vasospastic phenomena may be inferred for the intestine, and may escape clinical detection unless NEC occurs. In a study by Bunton and associates, catheterization of the umbilical artery was implicated as a significant predisposing factor for NEC [22], especially when catheterization was prolonged or complicated. Small emboli from indwelling aortic catheters pose a further risk, especially when the catheter tip is positioned high in the descending aorta above the orifices of the mesenteric arteries [23]. Tyson and associates found severe catheter related thromboatheromatous lesions at autopsy in 33 of 56 infants who had umbilical arterial catheters high in the aorta during life [24]. Major thrombosis of mesenteric arteries, however, is rarely documented in NEC [25]. Venous thromboemboli in the portal system may be implicated in cases of neonatal colonic perforation after exchange transfusion which is performed via a catheter in the umbilical vein [11–13, 26]. In some instances, hyperviscosity might initiate thrombosis [27].

Necrotizing enterocolitis has been associated with congenital heart disease although the postulated mechanisms have not been supported by clinical studies. Lowered perfusion pressure as a result of left ventricular outflow obstruction, such as coarctation, (PDA) or retrograde blood flow during diastole in patients with a patent ductus arteriosus [28], might lead to ischaemia of the bowel. Injury from hyperosmolar contrast media used during cardiac catheterization has also been implicated [29]. In a study of 133 babies with congenital heart disease Leung reported that 7% developed NEC. However, factors that correlated with NEC were prostaglandin E2 infusion, hypotensive episodes, and apnoea. There was no correlation with left ventricular outflow obstruction, PDA, or, cardiac catheterization [30]. A study from Indiana reported an increased risk of NEC in premature infants with PDA treated with indomethacin [31].

**Bacteria**

Necrotizing enterocolitis usually occurs during the first or second week after birth. It has not been described in stillborn infants. There may be a continuum between intestinal atresia, which is the most common congenital anomaly of the gastrointestinal tract, and NEC. If vascular insufficiency occurs prenatally in the sterile intestine, atresia can result. Postnatally, after the intestine is colonized with bacteria, the process is analogous to wet gangrene. Pathologic sections of late strictures of the intestine following NEC may bear a striking resemblance to sections from infants with atresia [32].

Bacterial colonization of the neonatal gut begins by contact with the vaginal flora and is propagated further by oral feedings and exposure to the environment. Normal infants are colonized with a range of aerobic and anaerobic flora by 10 days of age [33].

In the aseptic conditions of the NICU, however, infants undergo delayed colonization with a limited number of bacterial species [2, 34], which tend to be virulent. Hoy et al observed both a quantitative and qualitative change in the faecal flora prior to onset of NEC, with a decline in numbers of species and a preponderance of Enterobacteriaceae [35, 36].

Although an infectious etiology has been suspected, no common bacterium or virus has been isolated from infants with NEC. The organisms isolated are members of the normal flora of the neonatal gut, most commonly Klebsiella [37, 38], E. coli [37, 39] and Clostridia [40, 41]. Clostridium perfringens, which produces exotoxins, is associated with a fulminant, highly-lethal form of NEC [41, 42]. Cases of NEC usually occur sporadically, but are sometimes clustered in an epidemic form. Although a predominant organism may be isolated in such epidemics [37, 38], the documentation of asymptomatic carriers suggest that bacteria alone do not initiate the disease.
Feedings

Most infants who develop NEC have been fed a cow’s milk formula. Although hyperosmolar formulae have been implicated in a few instances [43, 44], the mechanism by which isosmolar milk formulas may lead to NEC has not been established.

Enteric bacteria, acting on formula as a substrate in the intestinal lumen, produce the blebs of pneumatosis intestinalis. Evidence for this mechanism was provided by analysis of the gas bubbles, showing that hydrogen predominated [45]. Conversely, pneumatosis is found in only 57% of unfed infants who develop NEC [46]. Some investigators believe that overfeeding premature infants in order to meet their high caloric requirements may contribute to the development of NEC, and advocate a restricted feeding schedule to decrease the incidence of NEC [47, 48]. In two prospective studies, however, there was no difference in the incidence of NEC between infants on a restrictive or rapidly increasing feeding schedule [49, 50].

NEC is rare among infants fed breast milk alone. In the animal model of Barlow et al [51], breast milk protected against NEC in newborn rats. In humans, breast milk contributes to the passive immunity of the neonatal intestine by providing macrophages, secretory immunoglobulin A, lactoferrin and other substances. The prospective multicentre study of Lucas and Cole showed that NEC was 6–10 times more common amongst formula fed babies than those fed breast milk alone, and three times more common in those who received formula plus breast milk [52].

The Lawrence hypothesis

In 1982, Lawrence et al [2] proposed an alternative hypothesis for the pathogenesis for NEC, based on abnormal bacterial colonization of infants in the NICU. These investigators demonstrated delayed gut colonization with limited numbers and species of bacteria. They postulated that the immature ileum, which absorbs bacterial toxins intact, sustained mucosal damage that initiated NEC. Evidence in support of this theory included Lawrence’s production of necrotic enteritis in a germ free neonatal rat model, by introduction of one of six toxin-forming bacteria. The rat model, however, inexplicably ceased to work about one year later [53].

Epidemiological studies have evaluated multiple predisposing factors, both maternal and neonatal, which were believed to be associated with NEC. Ryder et al [54] carried out an exhaustive epidemiologic study from 13 different centres which evaluated 400 possible predisposing factors in 111 patients and 112 controls. 390 variables did not correlate with NEC. The ten variables which appeared to correlate could have been the result of chance alone. Stoll et al [55] working in a single centre studied 113 variables in 35 cases and 98 controls. None correlated with NEC. The only consistent finding, in 89% of cases, was prematurity. Such studies generally support the Lawrence theory, which implicates a unique vulnerability of the intestine of preterm infants.

The immature intestine lacks secretory immunoglobulin A (IgA) which is important in the development of the mucosal barrier to invasion. Further, intestinal B and T lymphocytes are decreased in number in the newborn infant [56]. A study by Bell et al showed increased serum levels of IgA in infants with NEC, compared to a control group [57]. This might infer a decrease in secretory IgA at the mucosal border. Enhancement of the gastrointestinal host defence with human milk, immunoglobulin feeding, or corticosteroid administration may decrease the incidence of NEC. Lucas and Cole showed a decreased incidence of NEC among infants fed maternal breast milk [52]. Eibl used an oral preparation of IgA-IgG to reduce the incidence of NEC in infants at risk in Vienna [58]. Bauer et al identified a decreased incidence of NEC among babies born after prenatal corticosteroid therapy to prevent RDS [59]. Halac et al from Argentina, in a prospective study of both prenatal and postnatal corticosteroid therapy, found an incidence of NEC of 3.4% with prenatal steroid treatment and 6.9% with postnatal treatment, compared to 14.4% in the control group [60]. Substances which are trophic to the immature gut, or which function as nitric oxide donors, have shown promise in experimental models of bowel necrosis [61–63], but have yet to be tried clinically.

Multifactorial pathogenesis

Neither Santulli nor the Lawrence theory explains all cases of NEC. Ten percent of cases occur in term infants, usually considered at low risk for NEC. The syndrome may occur after the first two weeks of
life when gut colonization is usually complete [33] and the intestinal mucosa is no longer permeable to macromolecules [64]. NEC has been reported in infants fed breast milk exclusively [36] and in infants who were never fed at all [46, 65]. Most important, neither theory addresses the question of why NEC failed to develop among the majority of infants in the NICU environment, whose stress, colonization, feedings and immaturity are identical to the 2–4% of infants in the NICU who do develop the disease.

In an attempt to explain these phenomena, an hypothesis derived from the Santulli theory, was offered in 1984, as follows: NEC is caused by the coincidence of at least two of the three events: (1) intestinal ischaemia, (2) colonization of the gut by pathogenic bacteria and (3) excess protein substrate in the lumen. NEC is most likely to appear following quantitative extremes of these three elements, i.e., severe ischaemia, highly pathogenic flora, or a marked excess of substrate. NEC develops only if a threshold of injury, sufficient to initiate intestinal necrosis is exceeded [3]. The hypothesis was subsequently modified to consider the immunological immaturity of the preterm gut [4]. An experimental model was developed in germ free and gnotobiotic rats for evaluation of the comparative effects of ischaemia, bacteria and substrate [66]. In the pathogenesis of intestinal necrosis, the most important of the three factors was bacteria [66].

A mathematical model for NEC was then proposed, in an attempt to describe the contribution of various pathogenic factors to ischaemic bowel mass [5]. The concept of ischaemic mass differs from the usual clinical approach employed by neonatologists and paediatric surgeons, whose diagnostic efforts are directed to early identification of full thickness necrosis (gangrene), in order to carry out an emergency operation for resection of the gangrenous bowel. Clinical experience suggests the prognosis is far worse for an infant with extensive partial-thickness necrosis than for an infant with a short segment of frankly gangrenous bowel. Thus the ischaemic mass \( (M) \) was considered. The ischaemic mass \( (M) \) is directly proportional to length of injured intestine \( (L) \), depth of injury \( (D) \) and bacterial virulence factor \( (V) \). It is inversely related to the maturity of the mucosal barrier \( (m) \) and the time interval from onset of symptoms to the development of full thickness of necrosis \( (t) \). The hypothetical equation was proposed as follows:

\[
\frac{L \times D \times V}{m \times t}
\]

A quantitative scheme with categorical variables for each of the elements in the equation is outlined in Table 2. The data from a clinical series of 49 infants with stages II and III NEC by Bell’s classification [67], was substituted in the equation.

|   |   |
|---|---|
| **L** | Length of ischaemic intestine |
| 1 | Mild PI/≤15 cm gangrene |
| 2 | Moderate PI/15–40 cm gangrene |
| 3 | Severe PI/>40 cm gangrene |
| **D** | Depth of injury |
| 1 | Partial-thickness necrosis |
| 2 | Full-thickness necrosis (gangrene) |
| **V** | Bacterial virulence factor |
| 1 | Normal flora or no growth |
| 2 | Gram negative rod, not Klebsiella |
| 3 | Klebsiella |
| 4 | Clostridium perfringens |
| **m** | Maturity of intestinal barrier |
| 1 | Term |
| 0.75 | BA 34–37 weeks |
| 0.50 | GA 30–33 weeks |
| 0.25 | GA<30 weeks |
| **t** | Time from onset of NEC to intestinal gangrene |
| 1 | No evidence of gangrene |
| 0.50 | Gangrene ≥12 hours |
| 0.25 | Gangrene<12 hours |
Ischaemic mass ($M$) correlated with mortality from NEC (Fig. 1). The critical numerical value seemed evident at $M=48$. Mortality at this level was 50% (3 of 6). Below this level ($M<48$) mortality was 15% (4 of 17), whereas above this level ($M>48$) mortality was (12 of 15) 75%.

The numerical values assigned were arbitrary, and weighted for bacterial virulence factors ($V$) and immaturity of the intestinal barrier ($m$). The virulence factors were assigned on the basis of clinical observation [41, 42] plus the experimental work of Yale and Balish [68, 69]. Klebsiella, a pathogen commonly associated with NEC [38] was arbitrarily assigned a higher virulence number (three) than other Gram negative bacilli (two). The highest virulence number (four) was assigned to Clostridium perfringens which is responsible for a fulminant form of NEC, with an 87% mortality [41, 42]. The denominator considered the maturity of the intestinal barrier ($m$) and rapidity of the development of gangrene ($t$). Extreme immaturity and extremely rapid progression may have exponential effects on the ischaemic mass ($M$). Although enteral feeding may play a role in the pathogenesis of NEC, this was not included in the formula because trials had not shown a difference between the effect of a restricted or rapidly increasing intake of milk [49, 50]. More recent evidence of the benefits of breast milk [52] might justify the inclusion of feeding in the denominator, with breast milk as unity and formula feeding as some fraction thereof.

Because the pathogenesis of NEC is not completely understood, this equation includes factors which might be effects rather than causes, e.g., length of intestinal injury and time from onset to the development of gangrene. In the future, as neonatal pathophysiology is better understood, more elemental measurements may be substituted for such factors. New pathogenetic factors, as yet unknown, may exist for NEC, and will need to be appended to the equation.

**Further epidemiological considerations**

Although NEC is a serious neonatal problem in many countries in the world, its incidence is extremely low in Japan, Switzerland and some Scandinavian countries which have very low birth rates of preterm babies. In Shimura's survey of 52 centres in Japan, only 89 cases of NEC were identified from 1985–1989 among, 32,790 admissions of infants under 2500 grams (0.3%) [70]. The Japanese incidence was thus 10–20 times lower than that reported for the United States [7]. The nations favoured by a scarcity of NEC have some features in common: homogenous population, a high standard of living, administrative coherence, virtually universal access of health care, and high quality of prenatal care. If other developed countries could lower their preterm birth rate, NEC may decrease proportionately. Lowering the rate of preterm birth, however, is a formidable task, which may require social, economic or cultural change as well as improved access to prenatal care. There is a great need for research in this area of public health.

NEC occurs in two patterns: sporadic and epidemic. The bacteriology of sporadic NEC was prospectively studied in Melbourne [71]. No specific pathogenic bacteria were identified, and the organisms associated with NEC were members of the normal gut flora. Clostridium perfringens was associated with a clinically severe form of NEC. Clustering of cases, so called “epidemics” of NEC may sometimes occur, and may be linked with certain Enterobacteriaceae, particularly Klebsiella and E. coli [35, 37]. Two investigations which surveyed NICU flora over several months, documented that, when the prevalent intestinal flora changed from Klebsiella or E. coli to Serratia marcescens [72] or to Proteus mirabilis [35], NEC decreased or disappeared from the nursery. NEC may be associated with a specific viral or bacterial pathogen, such as Salmonella [72], rotavirus [74] or coronavirus [75]. The mechanism in these latter outbreaks is probably by mucosal injury during diarrhoea, which then permits invasion of the gut wall by enteric flora.

Finally, epidemiologic study of NEC must consider the sporadic and epidemic patterns of occurrence. In order to accurately determine the effect of
any intervention, the inclusion of simultaneous controls is mandatory. For example, an intervention which was introduced as an epidemic was subsiding could be credited with preventive powers, which, in fact, were false. To conclude on a lighter note, paradoxically, NEC sometimes seems to go away just as a prospective study of some kind is ready to start in an institution.

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