Neonatal necrotizing enterocolitis: a neonatal infection?

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Introduction

The condition known as neonatal necrotizing enterocolitis (NNEC) continues to excite the interest of paediatricians, microbiologists and infectious disease physicians for several reasons. Firstly, it has become recognized as an important cause of illness and death in neonatal intensive care units. Secondly, there are tantalizing suggestions that it has an infectious aetiology although no specific micro-organism has been convincingly shown to be the cause. Thirdly, there are a number of apparently analogous conditions in young animals which have been successfully controlled by anti-infective measures. Since there have recently been several general reviews of neonatal necrotizing enterocolitis (Kliegman & Fanaroff, 1984; de Louvois, 1986; Kliegman & Walsh, 1987), the focus of this article will be on the evidence which suggests that NNEC is the result of an infection. I will also consider what further experimental directions might improve the knowledge of the subject.

Clinical entity

First, it must be asked whether clinical descriptions of the patients are adequate to allow it to be considered a disease entity. If in fact it is only a complex of entities we should not be surprised to find difficulty in associating it with a specific micro-organism. There is general agreement on the complex of symptoms which suggest the diagnosis of NNEC. In 80–90% of the patients early events direct attention to the intestinal tract in an infant whose general condition suggests a serious infection. Abdominal distension is most common but occult intestinal bleeding is found in 60–80% of patients and one-third to one-half may have visibly bloody stools (Stevenson, Graham & Stevenson, 1980; Stoll *et al.*, 1980). These findings are very similar to those summarized in the 1981–82 British survey covering a total of 165 cases of either suspected or proven necrotizing enterocolitis.

The investigation indicated in a systemically ill infant who has abdominal distension and/or blood in the stools is a plain abdominal X-ray. Extra-mural gas or *pneumatosis intestinalis* is the hallmark of NNEC. Another finding very suggestive of the diagnosis is gas shadowing over the liver, representing gas in portal veins. *Pneumatosis intestinalis* is also a characteristic pathological finding in NNEC. Most of the gas-filled cysts are located in the submucosa and some of these cysts appear to be lined with cells suggesting that the gas collection has occurred within lymphatics. Necrotizing enterocolitis cases have been reported without *pneumatosis intestinalis*. Kliegman & Fanaroff (1982*a*) described 19 such patients, some of them presenting early in the course of their illness. Thirteen had evidence on X-ray of intestinal perforation without extra mural gas and five had ascites only. In summary, we may say that there are certain clinical findings which, when combined with *pneumatosis intestinalis* or evidence of intestinal perforation, describe a well-defined group of patients who will subsequently be found to have suffered intestinal necrosis.

Maternal effect

A number of authors have examined associated conditions or therapies in the mother or infant which have been associated with an increased risk of developing NNEC. Bunton *et al.* (1977) systematically used the case control method to compare affected infants with infants admitted just before and after the cases. Asphyxia and catheter related problems were significantly related to a risk of necrotizing enterocolitis. However, these authors observed that colitis did not develop until an average of 13 days after the umbilical artery catheters had been removed and the range was 2–29 days. Such an extended delay between catheter removal and development of symptoms vitiates any aetiological association. Stoll *et al.* (1980) also used a case control method but found none of the maternal and infant risk factors significantly associated. Kliegman *et al.* (1982*b*) compared clinical findings and risk factors in patients with the entire population of low birth weight infants admitted to the unit. Only placentia abruptio was significantly more frequent among the cases of NNEC. However, it was found in only 19% of the cases as opposed to 8% of the controls. Thus, if we accept the view that necrotizing enterocolitis is a clinical entity, placentia abruptio cannot be its cause. It is worth pointing out that there is a substantial chance of finding a single factor significantly associated with NNEC if enough factors are tested for simply on the basis of chance. Indeed, it is clear that no single risk factor can possibly be incriminated as a common aetiology in NNEC. Prematurity is not even a universal factor. Tait & Kealy (1979) described five cases in full-term infants at Kingston Hospital. Two of these infants had no associated risk factors, one weighing 3·7 kg and the other 4·1 kg at birth. Each of them had an apgar score at 1 min of 9. A reasonable conclusion to draw from the efforts to associate various risk factors with NNEC is that

risk factors describe the patient population in which these infants are found and do not indicate the aetiology of NNEC.

Evidence for infective cause

This leads us to the question, is neonatal necrotizing enterocolitis an infection? There are a number of lines of evidence. First, the disease has not been described in stillborn infants. This implies that bacterial colonization of the gastrointestinal tract is a prerequisite to its occurrence. Second, epidemiological studies support the infection hypothesis. Several large units describing the incidence of NNEC in their institutions over a number of years have noticed variations in the incidence of the disease from year to year or even from month to month. On a monthly basis, the aggregate number of cases may not be sufficient to allow statistical analysis. However, on a yearly basis there are sufficient numbers of cases to apply Chi-square analysis. If this is done for four large series published in the literature by Bell *et al.* (1979), Book *et al.* (1977), Kliegman & Fanaroff (1981), and Rotbart & Levin (1983) there is evidence of significant year to year variation (Table I). These series represent an average number of cases per year of between 11 and 28. The Bell series does not show significant year to year variation for a 6-year period but it has the fewest cases of any of the series. Both the Book and Kliegman series have significant year to year variations ($P < 0.01$). The series of Rotbart & Levin (1983), shows significant period-to-period variation ($P < 0.05$) if analysed on a 6-monthly basis. Non-random variation in prevalence of NNEC from year to year in several centres suggests that an environmental agent plays a role in the development of the disease.

Third, if NNEC is an infectious disease we might expect to find evidence of illness in medical and nursing staff who care for these patients. Gerber *et al.* (1985) calculated the relative risk of staff illness at various intervals between onset of illness in the staff member and exposure to disease in the neonate during an outbreak of NNEC. They found that for intervals of 1–4 days and 3–9 days there was a significant increased risk of illness in the staff member. However, these illnesses were not all related to the gastrointestinal tract. Some were, but some were syndromes of fever, nuchal rigidity and myalgia or fever and macular rash. The infants in this study were carefully

Table I. *Non-random prevalence of NNEC cases from year to year*

Series	Years	Average no. per year	Probability
Bell	1972–77	11	$< \cdot 1 \; > \cdot 05$
Book	1972–76	15	$< \cdot 01$
Kliegman	1972–78	16	$< \cdot 01$
Rotbart	1974–81	28	$< \cdot 1 \; > \cdot 05$
Rotbart	(6 monthly)	(14)	$< \cdot 05$

investigated for routine gastrointestinal pathogens including viruses and toxins and none were found.

A fourth argument for the infectious aetiology of NNEC might be successful antibiotic prophylaxis. Clinical trials of prophylaxis have been performed using both kanamycin and gentamicin. It has not been possible to confirm that prophylaxis is successful. The lack of such success is not strong evidence against an infectious aetiology. The success of antibiotic prophylaxis in other clinical settings is closely related to the accuracy with which the infecting organism can be predicted. An analogous argument concerns the prevention of neonatal necrotizing enterocolitis by the use of barrier nursing (Book *et al.*, 1977). However, barrier nursing has not been applied in a controlled manner and changes in the prevalence of the disease make historical controls unreliable.

Gas production is a fifth argument for microbial aetiology. *Pneumatosis intestinalis*, the hallmark of the disease, appears to consist of collections of hydrogen gas. The only possible source of hydrogen is anaerobic bacterial fermentation. However, it is not necessary that the hydrogen is formed from bacteria present in tissues. The solubility of hydrogen in water at standard pressure, 37°C is about the same as that of nitrogen. Both of these gases are less soluble in water than oxygen and very much less soluble than carbon dioxide. Unequal counter diffusion of gases within tissues can produce local areas of supersaturation with an increased risk of bubble formation at that site (D'Aoust & Lambertsen, 1982). The presence of gas does not require tissue invasion by bacteria, but extends the importance of the presence of bacteria even in the lumen. This leaves open the question of how tissue necrosis occurs.

Both viruses and bacteria have been candidate causes of NNEC. Cheny *et al.* (1982) reported seeing coronaviruses and Resta *et al.* (1985) were successful in cultivating gastrointestinal coronaviruses and in showing that they lack relationship with respiratory strains. However, neither of these studies showed coronaviruses to be associated with all cases of NNEC and many patients without intestinal lesions also have been excreting coronaviruses.

Klebsiella has been associated with NNEC in more than one report. None has been able to show that this organism was uniquely associated with the disease. The highest prevalence was reported by Westra-Meijer *et al.* (1983) who showed 71% of the NNEC patients colonized with klebsiella as compared with 37% of the controls. More recently, Blakey *et al.* (1985) found klebsiella in 44% of NNEC patients as compared with 26% of controls—not a significant difference. Westra-Meijer *et al.* (1983) identified five different serotypes of klebsiella with clustering of only one of the serotypes.

In a similar way, *Clostridium difficile* and *C. butyricum* have from time to time been found to be associated with the disease but these associations have failed to withstand further scrutiny. The most likely explanation for these

findings is that cross-colonization may indeed be occurring in units who experience cases of NNEC. This could account for the reported findings above, without implicating any organism as the aetiology. There is evidence both in patients and in animals that normal flora exert a protective effect in the bowel. Blakey *et al.* (1982) showed that neonatal intensive care units retard acquisition of normal flora and Lawrence, Bates & Gaul (1982) suggest that lack of normal flora could be associated with increased risk of super-infection or emergence of a pathogenic role for a micro-organism which normally is non-pathogenic.

Future research

Although the evidence adduced above argues for an infectious aetiology, it is clear that none of the organisms suggested have been shown to be causative. The question which then arises is, what could be done further to improve our knowledge of this disease. I would suggest first of all that it would be of use to study barrier nursing methods in a controlled manner. The physical layout of many neonatal intensive care units ought to permit such a study. Further use of routine cultural methods is not likely to be helpful. Limited efforts to detect toxins directly have been undertaken (Thomas, 1982). Unfortunately, there is no general method for toxin detection. Toxins might be inactivated *in situ* by intestinal or tissue proteases before being detected by any bioassay system. For example, *C. perfringens* alpha toxin is completely inactivated by 10 min of exposure to 1 mg ml^{-1} of chymotrypsin and it may, therefore, be necessary to use immunoassay methods to detect toxin fragments. Another highly sensitive technique would be the use of fluorescence substrates for toxins of known enzymatic specificity.

Conclusion

In this review I have defended the view that neonatal necrotizing enterocolitis is a clinical disease entity, and I have reviewed the evidence that bacteria play in a central role in its pathogenesis. Research directed at showing the presence of toxins directly in infected tissue might be a fruitful approach to further research in this subject.

Future research
Further use of routine cultural methods is not likely to be helpful as these have failed to substantiate a role in all cases even for *C. perfringens* (Kliegman *et al.*, 1979; Blakey *et al.*, 1985).

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