Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Gastrointestinal host defense and necrotizing enterocolitis

John N. Udall, Jr., MD, PhD

From the Department of Pediatrics, Children's Research Center, University of Arizona Health Sciences Center, Tucson, Arizona

Necrotizing enterocolitis is characterized by crepitant necrosis of the gut and occurs chiefly among stressed premature infants in neonatal intensive care units. The cause of NEC is unknown and there is no unifying etiologic theory.[1] Immaturity of gastrointestinal host defense most likely combines with ischemia, infection, and feeding to precipitate NEC. Although these predisposing factors are present in most premature infants, NEC develops in only 2% to 7% of premature infants.[2] A combination of events operating together at a critical point in time may be necessary to initiate the development of NEC. Alternatively, there may be other factors or influences not yet described that are important in initiating the process.

In this article I discuss gastrointestinal host defense mechanisms and suggest that immaturity of these defense mechanisms is the single most important factor in predisposing infants to NEC. In addition, evidence is presented to support the premise that the intestinal absorption of intact macromolecules and bacteria is common early in development; this aspect of intestinal immaturity increases the likelihood of NEC in premature infants. Finally, enhancement of gastrointestinal host defense with human milk, exogenous steroids, immunoglobulins, or antibiotics may decrease the incidence of NEC.

GASTROINTESTINAL HOST DEFENSE

Gastrointestinal host defense may be divided arbitrarily into immunologic and nonimmunologic defense mechanisms (Table I).

Immunologic gastrointestinal defense mechanisms. Intestinal B and T lymphocytes are decreased in number in the newborn infant. Accessory cells such as macrophages, mast cells, and Kupffer cells may be important to immunologic gastrointestinal host defense. However, these latter cell types have not been extensively studied to ascertain whether they change quantitatively or qualitatively during development.

B and T lymphocytes. Perkkio and Savilahti[3] noted decreased numbers of immunoglobulin-containing cells (B cells) in the intestinal mucosa of newborn humans compared with older infants. They suggested that a reduction in immunoglobulin-producing cells and secretory IgA in the intestine may account for the increased incidence of infection at mucosal surfaces early in life. Selner et al.[4] noted that only 10% of infants had detectable salivary IgA at 10 days of age. By 1 month of age, virtually 100% of infants born at term had detectable IgA in their saliva. Burgio et al.[5] also found decreased salivary IgA in infants compared with older children and adults. Secretory IgA, present in the intestine, is resistant to digestion and therefore is present in stool.[6] However, the antibody is present in only trace amounts in stool during the first few weeks of life compared with later in development (Figure 1).

BSA	Bovine serum albumin
LVP	Lysine vasopressin
MVM	Microvillus membranes
NEC	Necrotizing enterocolitis

Immunoglobulin A, which is present in human milk and contributes to the protective effect of milk, will be discussed more fully later. This immunoglobulin is produced in the intestine, by mucosal plasma cells that have differentiated from B lymphocytes.[7-9] The plasma cells, located in the lamina propria of the mucosa, synthesize dimeric IgA, which is composed of two IgA molecules (dimeric IgA) connected by a joint, or J piece.[10] A secretory component on the enterocyte membrane acts as a receptor for the dimeric IgA.[9] The secretory component has been localized by

Reprint requests: John N. Udall, Jr., MD, PhD, Department of Pediatrics, Arizona Health Sciences Center, 1501 N. Campbell Ave., Tucson, AZ 85724.

9/0/21256

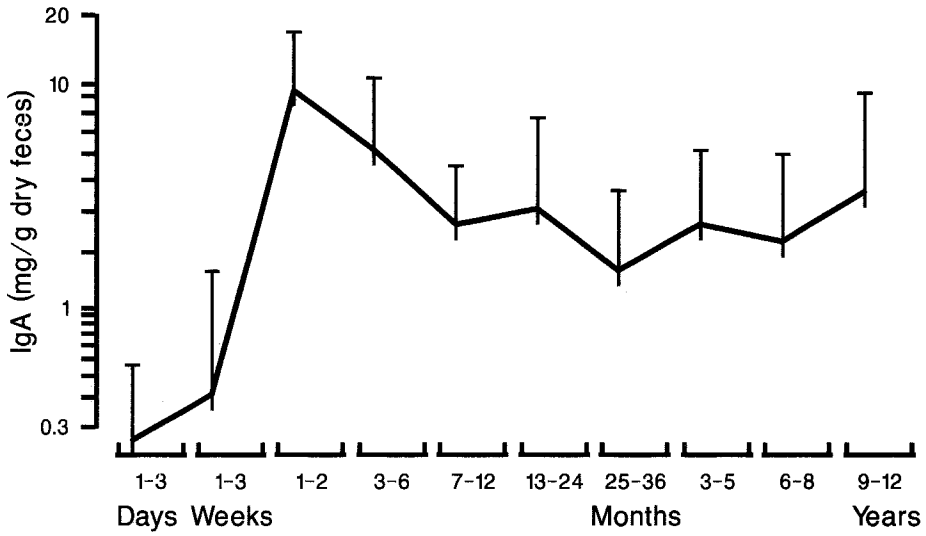

Figure. Mean concentration of IgA in extracts of single small samples of feces from infants and children of various age groups. Material collected at 1 to 3 days of age was meconium. Upper SD values are indicated by *vertical lines.* Number of persons in each group varied from 9 to 22. (From Brandtzaeg P, Baklien K, Bjerke K, Rognum TO, Scott H, Valnes K. Nature and properties of the human gastrointestinal immune system. In: Miller K, Nicklin S, eds. Immunology of the gastrointestinal tract; vol 1. Boca Raton, Fla.: CRC Press, 1987:1-85; Source, Blackwell Scientific Publications Limited.)

Table I. Gastrointestinal host defense

Immunologic mechanisms
Humoral immune system
B lymphocytes
Plasma cells
Cellular immune system
Helper T lymphocytes
Suppressor T lymphocytes
Killer T lymphocytes
Accessory cells
Macrophages
Mast cells
Kupffer cells
Nonimmunologic mechanisms
Gastric acid
Proteolytic enzymes
Intestinal mucin
Indigenous flora
Lysozyme
Bile salts
Peristalsis

immunohistochemistry and immunoelectron microscopy studies to the basolateral membranes of intestinal enterocytes and of epithelial cells lining exocrine glands. The receptor has also been localized on hepatocyte membranes of certain species, where it allows for the uptake and transport of IgA and IgA immune complexes through the hepatocytes into bile and then into the intestine.[9, 10]

Secretory IgA, produced in the intestine and excreted onto the mucosal surface, binds antigen, thereby preventing antigen attachment to the epithelial surface.[9, 10] It has been suggested that secretory IgA may prevent bacterial adhesion to the intestinal epithelial surface and suppress the growth of certain bacterial pathogens. Secretory IgA may also be important in enhancing resistance against viral enteritis. The quest for other potential actions of secretory IgA, such as opsonization and complement fixation, has been largely unsuccessful.

Necrotizing enterocolitis occurs predominantly in the ileum and proximal colon.[2] Large numbers of lymph follicles are present in the ileum and cecum. Pappo and Owen,[11] using rabbits, examined the location of IgA secretory component overlying lymph follicles in this region of the intestine. Using monoclonal antibodies to secretory component, they showed intense staining of the epithelial cells from regions between lymphoid patches, suggesting the presence of secretory piece associated with epithelial cells. However, no secretory component was identified on cells overlying the lymphoid follicles (Peyer patches). As cells emerged from intestinal crypts, there was a sharp demarcation of secretory component—positive cells from secretory component–negative cells. Those cells destined to populate the epithelium overlying Peyer patches had no detectable secretory component, whereas those destined to populate the villi did have secretory component. These findings demonstrate a unique difference in the expression of the receptor for IgA between

lymphoid-associated and non-lymphoid-associated epithelium. Increased macromolecular uptake[12-14] and the translocation of infectious agents across the intestine[15-19] may occur across lymphoid-associated epithelium in the distal ileum, where there are few IgA receptors.[11] These observations of decreased IgA over the Peyer patches of the ileum and increased macromolecular uptake in this area are interesting in view of the localization of NEC to this part of the intestine.

Investigators have documented decreased numbers of intestinal T lymphocytes in infant animals, indicating that cellular immaturity is also compromised early in life. Guy-Grand et al.,[20] studying a variety of mice strains, found that T lymphocytes were practically absent during the first 3 weeks of life. From day 21 to 35 of life, T lymphocytes in the intestinal mucosa gradually increased, and by day 50 the concentration had reached adult levels. Although subsets of helper and suppressor T lymphocytes within the intestine may enhance or suppress specific antibody production, information concerning intestinal T lymphocyte function is incomplete. It has been proposed that T cells present in epithelia mediate immunologic surveillance and monitor the integrity of epithelia.[21] The T cells may recognize alterations in the membrane of an epithelial cell infected by a bacterial or viral agent, and then destroy the infected cell before the infectious agent can cross the basement membrane underlying the epithelium.[21]

Effect of antigens, nutrition, and stress. Newborn infants, particularly premature infants, have a decreased ability to respond immunologically to a feeding of exogenous protein. Rieger and Rothberg[22] fed bovine serum albumin to human neonates of various gestational ages; four of five infants born at 35 weeks of gestation or later had antibodies to BSA, whereas none of eight infants born at less than 35 weeks of gestation produced anti-BSA. This finding raises the question of whether premature infants are less likely to respond immunologically to the uptake of exogenous toxins elaborated by bacteria important in the pathogenesis of NEC.

Normal antigenic exposure includes not only potential antigens present in infant formula but also bacterial proteins and peptides. Newborn and germ-free animals exposed to few antigens have a striking absence of antibody-producing cells in the intestine and little humoral response.[23-26] Antigen stimulation, then, is another important ingredient in normal intestinal immune development. Because of physical isolation, the cleanliness of nursing procedures, and the use of multiple antibiotics, the normal bacterial colonization of premature infants may be delayed. Limited antigenic exposure may retard intestinal immune development, and the organisms may then multiply in the gut unhindered. The immature gut does not suppress the bacteria, especially in the lower ileum, and toxic products from the growing bacteria may be absorbed and may cause mucosal damage, initiating NEC.[27]

Poor nutritional status may be present in premature infants, and has a marked adverse effect on systemic and intestinal immune function.[28-31] Spenger et al.[32] described 29 cases of NEC that occurred after the neonatal period; 22 of the 29 infants were malnourished (weight <80% of the expected weight for age). The authors suggested that the underlying malnutrition placed these infants at risk for the development of NEC.

Finally, stress imposed on newborn infants may interact with intestinal immune function and other predisposing factors. Although the "stress factor" is difficult to quantitate, Jemmott et al.[33] quantitated the effect of "academic stress" on immune function in 64 first-year dental students. The salivary IgA secretion rate was significantly lower in high-stress compared with low-stress periods. This study was performed in physically mature subjects and it is difficult to extrapolate the findings to stressed newborn infants. Nevertheless, it is possible that stress may interact with other predisposing factors to compromise intestinal immune function in newborn infants.

Nonimmunologic gastrointestinal defense mechanisms. Nonimmunologic gastrointestinal mechanisms enhance and augment intestinal host defense.

Gastric acid. Gastric acid secretion is a first line of defense against ingested pathogens and toxins.[34] Gastric acid must be at least partially neutralized with bicarbonate before some types of infectious diarrhea will develop in human volunteers. Music et al.[35] challenged healthy volunteers orally with toxin-producing *Vibrio cholerae.* Diarrhea was produced by 10^4 organisms when bicarbonate was administered simultaneously. The bicarbonate helped to neutralize gastric acidity; without it, a total of 10^8 organisms were necessary to induce diarrhea. The duration of the bicarbonate buffer effect could be correlated with susceptibility to disease induction. Kraft et al.[36] reported increased circulating anti-BSA antibodies in adults with achlorhydria and suggested that these persons do not adequately hydrolyze BSA present in milk and milk products. The protein is therefore present in greater concentrations in the intestine for absorption. The immune system is triggered by the increased antigen load, and antibodies are produced in increased amounts.

Gastric hydrogen ion output during the first month of life in human infants is markedly decreased compared with that in adults.[37] Therefore infants are at an increased risk for colonization of enteric pathogens, which may contribute to the development of NEC.

Proteolytic enzymes. The synthesis of proteolytic enzymes by the pancreas appears to be another important nonimmune intestinal defense mechanism. Walker et al.[38] showed that the breakdown of iodine 125–labeled BSA by jejunal gut sacs from pancreatic duct–ligated rats is significantly decreased in comparison with that in control animals with intact pancreatic ducts. The increased concentration of intact protein present when pancreatic proteases are blocked from entering the intestine is then available for absorption. Walker et al. found that the enteral treatment of pancreatic duct–ligated rats with pancreatic extracts led to an increase in the digestion of ^{125}I-BSA in the gut sacs in vitro and to a decrease in the absorption of intact BSA. Saffran et al.[39] fed lysine vasopressin and aprotinin (an inhibitor of trypsin) or LVP alone to adult Sprague-Dawley rats and noted an increased antidiuretic effect in LVP- and aprotinin-fed animals compared with animals given LVP alone. The authors suggested that inhibition of pancreatic proteases led to the transport of increased amounts of intact vasopressin across the intestine and into the blood to exert a systemic effect. Our experiments support the findings of Saffran et al. Newborn rabbits underwent gavage with BSA and aprotinin or with BSA alone[40]; animals treated with BSA and aprotinin had more than twice the amount of immunoreactive BSA per milliliter of plasma 4 hours after a gavage feeding than did animals given BSA alone. Others have suggested that in the suckling animal, intraluminal intestinal proteolytic activity is a regulator of nonselective protein uptake.[41]

Enteropeptidase (enterokinase), the brush-border enzyme present in the duodenum, converts inactive trypsinogen to active trypsin. Antonowicz and Lebenthal[42] found that full-term human infants had only 17% of the enteropeptidase (enterokinase) in intestinal washes that children 1 to 4 years of age had. This finding is in agreement with the observation that newborn infants have approximately one tenth of the intestinal tryptic activity found in children 9 months of age and older.[43]

That proteolytic activity is important in intestinal host defense is supported by studies of NEC in infants, children, and adults in New Guinea who subsist on a diet high in natural antiproteases.[44, 45] However, at periodic festivals, meat is eaten that may have been poorly cooked after contaminated with *Clostridium perfringens,* which produces a beta toxin. The infant, because of suppression of intestinal proteases and perhaps malnutrition, does not effectively hydrolyze the toxin, and the unhydrolyzed toxin produces severe inflammation of the intestine.[44, 45]

Intestinal mucins. Intestinal mucins are also important in intestinal host defense. These complex macromolecules contain a protein core and carbohydrate side chains. Mucin production takes place in both goblet cells and intestinal epithelial cells. Forstner[46] stated that a large part of intestinal mucin arises from epithelial cells. However, mucins from goblet cells, epithelial cells, and other glycoproteins probably all contribute to the mucin layer of the intestine. Edwards[47] suggested that intestinal mucins may be a medium in which most macromolecules are insoluble, whereas small molecules are soluble and free to diffuse. He speculated that the prime role of mucins may therefore be to exclude macromolecules and larger particles from contact with cell membranes. He pointed out that IgA is present with mucins in many secretions. In fact, IgAmolecules have a mucin-like glycoprotein sequence at the hinge region between their Fc and Fab subunits.[10, 48] This observation raises the possibility that IgA, with its mucin-like portion in the mucin phase, sits at the interface between mucin and overlying fluid. The remainder of the molecule extends into the overlying aqueous phase, like a detergent or a phospholipid at the interface between a surface phase and a water phase. The IgA would then form a monolayer on the surface of the mucin, where it is needed in defense against potential antigens and pathogens. Edwards' suggestion that mucin retards macromolecular absorption is supported by some experimental observations. Nimmerfall and Rosenthaler[49] noted that diffusion of a compound through the hydrated mucin network is likely to depend on the charge and hydration radius of the molecule and on the ability of the molecule to form hydrogen bonds. They found that intestinal absorption of molecules in the rat correlates inversely with molecular weight. Hence molecules of high molecular weight are retarded in their passage through the mucin layer and therefore are not absorbed as extensively as small molecules.

Shigella,[50] *Staphylococcus,*[51] *Klebsiella,*[52] and *Vibrio cholerae*[53] enterotoxins stimulate mucin release, synthesis, or both. The increased amount of mucin in response to an enterotoxin may be a protective mechanism of the host. Strombeck and Harrold[54] found that gastric and intestinal mucins bind and precipitate choleragen. The intestinal mucin blanket appears to be scant in newborn infants, perhaps facilitating bacterial adherence to the epithelium.[55, 56] In mice the mucin layer increases in thickness until the time of weaning.[56]

Other factors. It has been postulated that indigenous intestinal flora, intestinal secretions that contain lysozyme, bile salts, natural antibodies, and peristaltic movement may also be important in nonimmune intestinal host defense.

INTESTINAL PERMEABILITY

The neonate absorbs greater amounts of intact immunoglobulins, proteins, carbohydrates, and bacteria than does the adult. Although this increased permeability of the intestine may be considered the result of immature host defense mechanisms, the absorption of intact immunoglobu-

lins is a mechanism by which some newborn animals acquire passive immunity and thereby obtain protection from infectious agents.[57]

Absorption of intact immunoglobulins. Immunoglobulins present in milk are transported across the intestine of suckling goats, pigs, cows, and horses.[58] The guinea pig, rabbit, monkey, and human being acquire maternal immunoglobulins prenatally in utero. The cat, dog, mouse, and rat receive immunoglobulins both in utero and postnatally.[58]

Early investigators were convinced that immunoglobulins were not absorbed intact from human milk into the circulation of human infants.[58-60] However, more recent studies oppose this view.[61-65] Iyengar and Selvaraj[61] quantitated serum immunoglobulins from birth to 5 days of age. The serum concentration of immunoglobulins A, G, and M on day 5 was significantly higher in the serum of colostrum-fed human infants than in exclusively bottle-fed infants. The investigators suggested that immunoglobulins present in colostrum are to some extent absorbed intact from the intestine of human milk–fed infants. Vukavic[62, 63] confirmed their observation by quantitating the concentration of IgA in human infants fed intact IgA and IgA denatured by heating; the denatured IgA was not recognized by the immunodiffusion assay used. Infants fed intact IgA had significantly increased serum IgA concentrations in comparison with infants fed denatured IgA. Roberts and Freed[64] measured the IgA concentration in the saliva and nasal mucus of breast-fed and bottle-fed infants; breast-fed infants had significantly increased IgA levels compared with bottle-fed infants, and the authors suggested that the IgA-producing lymphocytes of the neonate are "switched on" by a factor in maternal colostrum. Ogra et al.[65] administered to infants human colostrum rich in IgA directed against polio virus. All infants at birth had umbilical cord blood without antipolio antibodies. However, when seven infants whose ages ranged from 18 to 72 hours were fed the colostrum, detectable antipolio IgA was present in the serum of three infants shortly after the feeding. The authors concluded that intact IgA may have been transported across the intestine of these infants to convey passive immunity. The evidecnce now suggests that human milk is associated with absorption of intact IgA or stimulation of plasma cells to produce increased amounts of IgA, or both.

Absorption of intact proteins. We have quantitated the absorption of intact nonimmunoglobulin proteins in developing animals. Newborn rabbits were given BSA by orogastric feeding.[66, 67] Blood obtained 4 hours after the gavage contained approximately 5 μg of immunoreactive BSA per milliliter of plasma (Table II). Weaned animals given the same amount of BSA per unit of body weight did not have detectable immunoreactive BSA in plasma after the feeding. Even when blood was obtained from older animals at 1, 2, 4, 8, and 16 hours after the BSA feeding we were unable to detect immunoreactive BSA in the plasma of the mature animals by means of electroimmunodiffusion. However, with a more sensitive radioimmunoassay, nanogram quantities of BSA were detected in the plasma of the older animals. Newborn animals also had an increased clearance of plasma BSA compared with adult animals.[66] We concluded that the increased levels of immunoreactive BSA in the plasma of newborn animals after BSA feeding did not represent a decrease in the clearance of the intravascular protein but, instead, represented increased absorption of intact BSA.

Table II. Plasma concentration of immunoreactive bovine serum albumin in rabbits 4 hours after feeding with bovine serum albumin

Age	Dose of BSA (mg/100 gm BW)	D/T	iBSA (μg/ml plasma)
1 day (suckling)	200	8/9	5.5 ± 1.5*
1 wk (suckling)	100	14/15	6.1 ± 0.8
2 wks (suckling)	200	5/5	3.8 ± 0.5
4 wk (weaning)	200	0/10	ND
52 wk (weaned)	100	0/2	ND

From Udall JN, Block KJ, Walker WA. Lancet 1982;1:1441-3. Reproduced by permission.

BW, body weight; *D/T*, number of animals with detectable iBSA/number of animals tested; *iBSA*, immunoreactive BSA; *ND*, not detected.
*Values are expressed as mean ± SEM.

Studies of human infants have led to the same conclusion; the intestine is more permeable to the uptake of intact proteins early in life than in adulthood. Infants and children have increased amounts of antibodies to cow milk protein in their serum compared with adults.[68, 69] The assumption is that the increased levels of cow milk protein antibodies are due to the increased transport of intact protein across the intestine and subsequent stimulation of the systemic immune system. Roberton et al.[70] reported more direct evidence that intact protein absorption occurs in human infants. They measured the concentration of β-lactoglobulin in the sera of 47 preterm and term infants after feeding a standard amount of a cow milk–based formula. Preterm infants, particularly those born at less than 33 weeks of gestation, had higher serum concentrations of β-lactoglobulin than did term neonates given an equivalent milk feeding. These results suggest that the ability of the gastroinestinal tract to exclude antigenically intact food proteins increases with gestational age.

Absorption of intact carbohydrates. Beach et al[71] presented direct evidence that the permeability of the intestine of preterm human infants to intact carbohydrates is greater than in children and adults. They determined the urinary

excretion of lactulose and rhamnose after the ingestion of milk containing the carbohydrates. Infants born at a gestational age of 31 to 36 weeks had a period of enhanced permeability to lactulose during the first week of life. The lactulose/rhamnose excretion ratio was significantly higher on day 2 than on days 9 or 16, when a mature pattern of permeability was seen. Infants born at a gestational age of 26 to 29 weeks had a "mature" pattern of permeability at birth, followed by a temporary period of enhanced permeability at 3 to 4 weeks of age. The authors proposed that the enhanced permeability to larger molecules is a temporary condition of the neonatal bowel in man, as in other mammals, but that the immunologic implications "remain to be established." Weaver et al.[72] found that babies born before a gestational age of 34 weeks had higher intestinal permeability to lactulose than more mature babies. The absorption of intact lactulose decreased appreciably during the first week. Weaver et al.[73] found that breast-feeding was associated with decreased intestinal permeability to intact lactulose compared with bottle feeding. Using an animal model,[74] we have observed decreased macromolecular absorption associated with breast-feeding, but others have shown that breast- and bottle-fed infants do not differ in intestinal permeability to intact macromolecules.[70]

The development of NEC has been associated with ischemia, infectious agents, and hyperosmolar feedings, as noted elsewhere in this symposium. The uptake of macromolecules across the intestine is increased during ischemia,[75] increased with certain infections,[76] and increased in the presence of hyperosmolar fluids.[77, 78]

Absorption of intact bacteria. Not only is the intestine of newborn infants more permeable to the uptake of macromolecules, it is also more permeable to the uptake of intact bacteria. When the mucosal barrier to the uptake of intact viable bacteria fails, indigenous bacteria colonizing the gastrointestinal tract may pass across the intestinal mucosa to infect the mesenteric lymph nodes and other systemic organs and tissues. This process is termed bacterial translocation. The translocation of intestinal bacteria seems more likely to occur in young animals.[79] In studies by Glode et al.,[80] orally administered *Escherichia coli* caused a 53% incidence of bacteremia in 2- to 3-day-old rats; the incidence decreased to 10% at 15 days of age and to zero at 30 days of age. Likewise, Pluschke et al.[81] studied strains of *E. coli;* almost all strains translocated to the blood or mesenteric lymph nodes when orally inoculated into newborn rats given no prior antibiotic therapy. The extent and ease of translocation of these organisms would not have been possible in adult animals.[79, 81]

Mizrahi et al.[82] described 18 premature babies who died of NEC. Enteric organisms were obtained from blood in 7 of 13 who were tested while alive. Postmortem blood samples were positive for enteric organisms in 13 of 16 infants tested. It is reasonable to conclude that translocation of enteric bacteria occurred in these infants during the later stages of NEC.

Although in most studies of macromolecular and bacterial uptake researchers have assumed that uptake takes place in the small intestine, recent data suggest that the colon is more permeable to macromolecules in the neonatal period than later in development. Newborn rabbits absorb from the colon and excrete in their urine significantly more polyethylene glycol than older animals.[83] This observation is of interest because NEC commonly affects the proximal portion of the colon.

ENHANCEMENT OF INTESTINAL HOST DEFENSE AND RISK OF NECROTIZING ENTEROCOLITIS

There is ample evidence that both immune and nonimmune intestinal host defense mechanisms are immature early in life and that this immaturity leads to the increased uptake of macromolecules and infectious agents. Intestinal immaturity puts the newborn infant, especially if born prematurely, at an increased risk for the development of NEC. However, the intestinal host defense of infants may be bolstered by the use of human milk, steroids, exogenous immunoglobulins, and antibiotics.

Human milk. Human milk may offer animals[84, 85] and human beings[86] partial protection from the development of NEC because of secretory IgA or phagocytic cells present in the milk. Immunoglobulin A may agglutinate with antigens, endotoxin, or bacteria to restrict their mucosal penetration. In human subjects with IgA deficiency, the uptake of macromolecules across the intestine is increased.[87, 88]

The IGA-producing plasma cells present in human milk derive their origin from the precursor immunocompetent cells in the gut-associated and bronchial-associated lymphoid tissue. It is believed that exposure of IgA precursor B lymphocytes to microbial and dietary antigens is an important prerequisite for B lymphocyte activation and proliferation.[89] Such antigen-sensitized cells are eventually transported via the systemic circulation to other mucosal surfaces, including the mammary glands. The cells then initiate synthesis of immunoglobulins against specific antigens previously present in the mucosa of the respiratory and alimentary tracts. Cells important in cellular immunity and components of cellular immunity also are absorbed from human milk in the neonatal period.[65] Specific antibody and cellular immune reactivity against many bacteria, viruses, and ingested food proteins have been repeatedly demonstrated in fresh human milk.[89]

Despite these apparent advantages, the protective effect

of milk in mitigating the incidence of NEC has been questioned.[2] To evaluate conclusively the effect of human milk and formula feeding on the development of NEC would require a large, prospective, randomized, multicenter study of infants at risk for NEC. Fresh human milk not exposed to glass or refrigeration would have to be compared with formula.

Exogenous steroids. Bauer et al.,[90] in a large multicentered collaborative randomized tiral, found a significantly decreased ($p = 0.002$) incidence of NEC in the infants whose mothers had been treated with steroids antenatally. Accelerated intestinal maturation induced by antenatal maternal steroid therapy may have accounted for the decreased incidence of NEC in steroid-exposed infants. There were no adverse reactions to steroid therapy. Glucocorticoids induce precocious maturation of the gut.[91] Celano et al.[92] showed that the antenatal administration of steroids to pregnant rats accelerated maturation of the fetal intestine; rats exposed in utero to exogenous steroids had a precocious appearance of jejunal sucrase. Administration of glucocorticoids to suckling rats or mice also increases salivary amylase activity, gastric acid secretion, and activity of gastric pepsinogen, pancreatic amylase, and a variety of other enzymes.[91]

Other studies have documented a change in intestinal microvillus membranes in young animals exposed to steroids. The MVM obtained from the intestine of newborn animals are disorderd in comparison with MVW from adult animals; this difference in membrane organization may account in part for the increased attachment and penetration of macromolecules noted during the perinatal period.[93-95] In addition, intestinal MVM from suckling animals contain greater amounts of total lipid, cholesterol, and phospholipid per miligram of protein than do mature MVM preparations.[94, 95] However, when pregnant rats are injected with dexamethasone, the intestinal MVM from their pups are more ordered, or mature, than those of matched control subjects.[96] Glucocorticoids administered postnatally also accelerate maturational changes in MVM.[96] The events of "closure" (the physical process of sealing the epithelial barrier early in life to the increased transport of macromolecules) appear to be preprogrammed and associated with intestinal maturation; evidence is contradictory as to the importance of steroids in accelerating closure.

Exogenous immunoglobulins. Blum et al.[97] fed 10% human IgG (1 to 8 ml/kg/day) to six premature infants; a seventh infant was not fed the globulin. Undigested and partially digested IgG was detected in the stools in significant quantities in all six infants fed the immune globulin but not in the control infant. The authors speculated that the newborn infant's intestinal enzymatic immaturity or the rapid transit time, or both, permitted the passage of intact or partially digested IgG through the entire gastrointestinal tract.

A milk immunoglobulin concentrate containing antibodies to enteropathogenic *E. coli* stains augments intestinal host defense in human infants.[98] The concentrate was prepared by hyperimmunizing pregnant cows and then obtaining milk from the cows during the first 6 to 8 days of lactation. The concentrate contained protein and immunoglobulin (35% to 40%). The antibacterial activity of the concentrate was measured by bacterial passive agglutination, bacteriostatic activity in vitro, phagocytic clearance in vivo, and a protection test in mice. Sixty infants and children (aged 10 days to 18 months) with diarrhea caused by enteropathogenic *E. coli* were treated for 10 days with the immunoglobulin concentrate, and stool cultures were grown before, during, and after treatment. The findings indicated that the ingestion of immunoglobulins with antibody specific to certain pathogens tends to protect infants from infection with these organisms.

In another study, lyophilized cow milk immunoglobulins were prepared from the colostrum of cows immunized with several enterotoxigenic *E. coli* serotypes.[99] Adult volunteers received the immunoglobulin concentrate against enterotoxigenic *E. coli* daily, and control subjects received a similar amount of concentrate with no activity against *E. coli*. On the third day, all were given the enterotoxigenic *E. coli*. None of the volunteers receiving the concentrate against *E. coli* had diarrhea subsequently, but 9 of the 10 control subjects did ($p < 0.0001$). All volunteers excreted the pathogenic *E. coli* in the stool. It was concluded that lyophilized cow milk immunoglobulins may be an effective prophylaxis against specific pathogens.

Recently the efficacy of a human immunoglobulin preparation (73% IgA, 26% IgG) in reducing the incidence of NEC in infants of low birth weight was tested.[100] There were no cases of NEC among the 88 infants receiving oral IgA-IgG, in comparison with six cases among the 91 control infants ($p = 0.014$). The authors concluded that the oral administration of IgA-IgG to low birth weight infants may prevent the development of NEC.

Antibiotics. It has been proposed that oral vancomycin may be used in the treatment or the prevention of NEC. Han et al.[101] studied an outbreak of NEC in a neonatal intensive care unit. Fifty-seven perinatal and neonatal factors, many of which were known to be associated with NEC, were compared between infants with NEC and unaffected infants admitted concurrently. *Clostridium difficile* cytotoxin was detected in the stools of 92% of affected infants and in 12% of control infants ($p < 0.001$). The organism was isolated in 62% of affected neonates and in none of the control infants ($p < 0.001$). The outbreak was terminated after oral vancomycin therapy and strict antiinfective measures were insti-

tuted in the neonatal intensive care unit. The authors suggested that vancomycin therapy is indicated for NEC outbreaks in units where *C. difficile* is endemic.

A series of studies in the 1970s sought to determine whether the prophylactic use of nonabsorbable antibiotics could reduce the incidence of NEC.[102-105] The results of these studies were contradictory. More recently, 84 very low birth weight babies considered at an increased risk for the development of NEC were given vancomycin orally for 18 hours before the introduction of oral feedings.[106] NEC developed in one infant. One hundred twenty very low birth weight babies not considered at increased risk for NEC were fed without first receiving vancomycin; NEC developed in 17 of them. Although this study was neither randomized nor controlled, the results suggest that vancomycin may have a place in prophylaxis against NEC in certain situations.

The administration of steroids or immunoglobulins, or both, to bolster the intestinal host defense of infants at risk for NEC may be of value. This approach may be more reasonable than the use of antibiotics, because several outbreaks of NEC have been associated with viral pathogens.[107-109] However, additional studies are necessary to evaluate the efficacy of these prophylactic regimens.

FUTURE STUDIES

Cell membranes. Recently it has been suggested that there is continual "cell membrane wounding and healing" of normal intestinal cells. McNeil and Ito[110] showed that intestinal cells of animals wounded in vivo by mechanical forces are capable of resealing disruptions of their plasma membranes. The wounding of cell membranes, followed by resealing, occurred not only in the mechanically injured gut but also in normal, experimentally undisturbed intestine. In the undisturbed gut, cell membrane wounding and resealing were most frequently observed in the colon but were also observed in the stomach and small intestine. If, indeed, epithelial cells are continually traumatized only to reseal, perhaps the infant at risk for NEC has decreased ability to reseal rapidly wounded enterocyte membranes.

Identification of infants at risk. Wilson et al.[111] studied the epidemiologic interrelationships among birth weight, gestational age, and age at onset of NEC. Weekly birth weight–specific attack rates for NEC declined sharply in all birth weight groups when the equivalent of 35 to 36 weeks of gestational age was reached. The authors' data are consistent with the hypothesis that the risk period for NEC is determined primarily by the *maturity of the gastrointestinal tract.*[111] These data are supported by other studies whose findings suggest that the occurrence of NEC is most closely associated with immaturity of the gastrointestinal tract and that *with increased maturatino there is a significantly lower incidence of NEC.*[112-117]

It appears reasonable to suggest that the development of NEC is dependent on the interplay of numerous factors. The premature infant is at greatest risk by virtue of immature intestinal host defense mechanisms, and when additional insults are introduced, such as stress (ischemia, cold), potential pathogens, and hyperosmolar feedings, the additional insults may overwhelm the immature intenstine. It is imperative to continue our studies of NEC to determine how we can best indentify infants at greatest risk for this disease. Additional research is also needed to evaluate whether gastrointestinal host defense may be bolstered in these infants to protect them from the development of NEC.

I thank Professor Otakar Koldovský for his critical review of the manuscript and my wife for editorial assistance.

REFERENCES

1. Kosloske AM. Pathogenesis and prevention of necrotizing enterocolitis: a hypothesis based on personal observation and a review of the literature. Pediatrics 1984;74:1086-92.
2. Kliegman RM, Walsh MC. Neonatal necrotizing enterocolitis: pathogenesis, classification, and spectrum of illness. Curr Probl Pediatr 1987;17:219-88.
3. Perkkio M, Savilahti E. Time of appearance of immunoglobulin-containing cells in the mucosa of the neonatal intestine. Pediatr Res 1980;14:953-5.
4. Selner JC, Merrill DA, Claman HN. Salivary immunoglobulin and alubmin: development during the newborn period. J PEDIATR 1968;72:685-9.
5. Burgio GR, Lanzavecchia A, Plebani A, Jayakar S, Ugazio AG. Ontogeny of secretory immunity: levels of secretory IgA and natural antibodies in saliva. Pediatr Res 1980;14: 1111-4.
6. Brandtzaeg P, Baklien K, Bjerke K, Rognum TO, Scott H, Valnes K. Nature and properties of the human gastrointestinal immune system. In: Miller K, Nicklin S, eds. Immunology of the gastrointestinal tract; vol 1. Boca Raton, Fla.: CRC Press, 1987:1-85.
7. Heremans JF. Immunoglobulin A. In: Sela M, ed. The antigens; vol 2. New York: Academic Press, 1974:365-522.
8. Pabst R. Review article: the anatomical basis for the immune fuction of the gut. Anat Embryol (Berl) 1987;176:135-44.
9. Kilian M, Mestecky J, Russell MW. Defense mechanisms involving Fc-dependent functions of immunoglobulin A and their subversion by bacterial immunoglobulin A proteases. Microbiol Rev 1988;52:296-303.
10. Crago SS, Tomasi TB. Mucosal antibodies. In: Brostoff J, Challacombe SJ, eds. Food allergy and intolerance. London: Bailliere Tindall, 1987:167-89.
11. Pappo J, Owen RL. Absence of secretory component expression by epithelial cells overlying rabbit gut–associated lymphoid tissue. Gastroenterology 1988;95:1173-7.
12. Owen RL, Jones AL. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. Gastroenterology 1974;66:189-203.
13. Owen RL. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study. Gastroenterology 1977;72:440-51.

14. Keljo DJ, Hamilton JR. Quantitative determination of macromolecular transport rate across intestinal Peyer's patches. Am J Physiol 1983;244:G637-44.
15. Wolf JL, Rubin DH, Finberg R, et al. Intestinal M cells: a pathway for entry of reovirus into the host. Science 1981; 212:471-2.
16. Inman LR, Cantey JR. Specific adherence of *Escherichia coli* (strain RDEC-1) to membranous (M) cells of the Peyer's patch in *Escherichia coli* diarrhea in the rabbit. J Clin Invest 1983;71:1-8.
17. Berg RD, Garlington AW. Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in the gnotobiotic mouse model. Infect Immun 1979;23:403-11.
18. Berg RD. Inhibition of *Escherichia coli* translocation from the gastrointestinal tract by normal cecal flora in gnotobiotic or antibiotic-decontaminated mice. Infect Immun 1980; 29:1073-81.
19. Deitch EA, Winterton J, Berg R. Thermal injury promotes bacterial translocation from the gastrointestinal tract in mice with impaired T-cell–mediated immunity. Arch Surg 1986; 121:97-101.
20. Guy-Grand D, Griscelli C, Vassalli P. The mouse gut T lymphocyte, a novel type of T cell: nature, origin, and traffic in mice in normal and graft-versus-host conditions. J Exp Med 1978;148:1661-77.
21. Janeway CA Jr, Jones B, Hayday A. Specificity and function of T cells bearing gamma/delta receptors. Immunol Today 1988;9:73-6.
22. Rieger CHL, Rothberg RM. Development of the capacity to produce specific antibody to an ingested food antigen in the premature infant. J PEDIATR 1975;87:515-8.
23. Abrams GD. Effects of the normal flora on the host defenses against microbial invasion. Adv Exp Med Biol 1969;3:197-206.
24. Crabbe PA, Nash DR, Bazin H, Eyssen H, Heremans JF. Immunohistochemical observations on lymphoid tissues from conventional and germ-free mice. Lab Invest 1970;22:448-57.
25. Ferguson A, Parrott DMV. The effect of antigen deprivation on thymus-dependent and thymus-independent lymphocytes in the small intestine of the mouse. Clin Exp Immunol 1972;12:477-88.
26. Pollard M, Sharon N. Responses of the Peyer's patches in germ-free mice to antigenic stimulation. Infect Immun 1970; 2:96-100.
27. Lawrence G, Bates J, Gaul A. Pathogenesis of neonatal necrotising enterocolitis. Lancet 1982;1:137-9.
28. Chandra RK. Immunocompetence in undernutrition. J PEDIATR 1972;81:1194-200.
29. Sirisinha S, Suskind R, Edelman R, Asvapaka C, Olson RE. Secretory and serum IgA in children with protein-calorie malnutrition. Pediatrics 1975;55:166-70.
30. Worthington BS, Syrotuck J. Intestinal permeability to large particles in normal and protein-deficient adult rats. J Nutr 1976;106:20-32.
31. Deitch EA, Winterton J, Berg R. Effect of starvation, malnutrition and trauma on the gastrointestinal tract flora and bacterial translocation. Arch Surg 1987;122:1019-24.
32. Spenger KJ, Kaschula ROC, Nicholson N. Necrotizing enterocolitis after the neonatal period. J Trop Pediatr 1987; 33:233-8.
33. Jemmott JB III, Borysenko JZ, Bozysenko M, et al. Academic stress, power motivation, and decrease in secretion rate of salivary secretory immunoglobulin A. Lancet 1983;1: 1400-2.
34. Udall JN. Maturation of intestinal host defense: an update. Nutr Res 1981;1:399-418.
35. Music SI, Libonati JP, Wenzel RP, Snyder MJ, Hornick RB, Woodward TE. Induced human cholera. Antimicrob Agents Chemother 1970;10:462-6.
36. Kraft SC, Rothberg RM, Knauer CM, Svoboda AC Jr, Monroe LS, Farr RS. Gastric acid output and circulating antibovine serum albumin in adults. Clin Exp Immunol 1967;2:321-30.
37. Grand RJ, Watkins JB, Torti FM. Development of the human gastrointestinal tract. Gastroenterology 1976;70:790-810.
38. Walker WA, Wu M, Isselbacher KJ, Bloch KJ. Intestinal uptake of macromolecules. IV. The effect of pancreatic duct ligation on the breakdown of antigen and antigen-antibody complexes on the intestinal surface. Gastroenterology 1975; 69:1223-9.
39. Saffran M, Franco-Saenz R, Kong A, Papahadjopoulos D, Szoka F. A model for the study of the oral administration of peptide hormones. Can J Biochem 1979;57:548-53.
40. Udall JN, Bloch KJ, Vachino G, Feldman P, Walker WA. Development of the gastrointestinal mucosal barrier. IV. The effect of inhibition of proteolysis on the uptake of macromolecules by the intestine of the newborn rabbit before and after weaning. Biol Neonate 1984;45:289-95.
41. Telemo E, Westrom BR, Karlsson BW. Proteolytic activity as a regulator of the transmission of orally fed proteins from the gut to the blood serum in the suckling rat. Biol Neonate 1982;41:85-93.
42. Antonowicz I, Lebenthal E. Developmental pattern of small intestinal enterokinase and disaccharidase activities in the human fetus. Gastroenterology 1977;72:1299-303.
43. Zoppi G, Andreotti G, Pajno-Ferrara F, Njai DM, Gaburro D. Exocrine pancreas function in premature and full-term neonates. Pediatr Res 1972;6:880-6.
44. Murrell TGC, Roth L, Egerton J, Samels J, Walker PD. Pigbel: enteritis necroticans—a study in diagnosis and management. Lancet 1966;1:217-22.
45. Lawrence G, Shann F, Freestone DS, Walker PD. Prevention of necrotising enteritis in Papua New Guinea by active immunisation. Lancet 1979;1:227-30.
46. Forstner JF. Intestinal mucins in health and disease. Digestion 1978;17:234-63.
47. Edwards PAW. Is mucus a selective barrier to macromolecules? Br Med Bull 1978;34:55-6.
48. Liu YSV, Low TLK, Infante A, Putnam FW. Complete covalent structure of a human IgA1 immunoglobulin. Science 1976;193:1017-20.
49. Nimmerfall F, Rosenthaler J. Significance of the goblet-cell mucin layer, the outermost luminal barrier to passage through the gut wall. Biochem Biophys Res Commun 1980;94:960-6.
50. Keusch GT, Grady GF, Takeuchi A, Sprinz H. The pathogenesis of shigella diarrhea. II. Enterotoxin-induced acute enteritis in the rabbit ileum. J Infect Dis 1972;126:92-5.
51. Sheahan DG, Jervis HR, Takeuchi A, Sprinz H. The effect of staphylococcal enterotoxin on the epithelial mucosubstances of the small intestine of Rhesus monkeys. Am J Pathol 1970;60(1):1-18.
52. Klipstein FA, Schenk EA. Enterotoxigenic intestinal bacte-

ria in tropical sprue. II. Effect of the bacteria and their enterotoxins on intestinal structure. Gastroenterology 1975;68:642-55.
53. Sherr HP, Mertens RB, Broock. Cholera toxin–induced glycoprotein secretion in rabbit small intestine. Gastroenterology 1979;77:18-25.
54. Strombeck DR, Harrold D. Binding of cholera toxin to mucins and inhibition by gastric mucin. Infect Immun 1974; 10:1266-72.
55. Allen A. Structure and function of gastrointestinal mucus. In: Johnson LR, ed. Physiology of the gastrointestinal tract; vol 1. New York: Raven Press, 1981:617-39.
56. Rozee KR, Cooper D, Lam K, Costerton JW. Microbial flora of the mouse ileum mucous layer and epithelial surface. Appl Environ Microbiol 1982;43:1451-63.
57. Udall JN, Walker WA. Medical progress: the physiologic and pathologic basis for the transport of macromolecules across the intestinal tract. J Pediatr Gastroenterol Nutr 1982;1:295-301.
58. Kraehenbuhl JP, Campiche MA. Early stages of intestinal absorption of specific antibodies in the newborn: an ultrastructural, cytochemical and immunological study in the pig, rat and rabbit. J Cell Biol 1969;42:345-65.
59. Ammann AJ, Stiehm ER. Immune globulin levels in colostrum and breast milk, and serum from formula- and breast-fed newborns. Proc Soc Exp Biol Med 1966;122:1098-100.
60. Kenny JF, Boesman MI, Michaels RH. Bacterial and viral coproantibodies in breast-fed infants. Pediatrics 1967;39:202-13.
61. Iyengar L, Selvaraj RJ. Intestinal absorption of immunoglobulins by newborn infants. Arch Dis Child 1972;47:411-4.
62. Vukavic T. Intestinal absorption of IgA in the newborn. J Pediatr Gastroenterol Nutr 1983;2:248-51.
63. Vukavic T. Timing of gut closure. J Pediatr Gastroenterol Nutr 1984;3:700-3.
64. Roberts SA, Freed DLJ. Neonatal IgA secretion enhanced by breast feeding [Letter]. Lancet 1977;2:1131.
65. Ogra SS, Weintraub D, Ogra PL. Immunologic aspects of human colostrum and milk. III. Fate and absorption of cellular and soluble components in the gastrointestinal tract of the newborn. J Immunol 1977;119:245-8.
66. Udall JN, Pang K, Fritze L, Kleinman R, Walker WA. Development of gastrointestinal mucosal barrier. I. The effect of age on intestinal permeability to macromolecules. Pediatr Res 1981;15:241-4.
67. Udall JN, Bloch KJ, Walker WA. Transport of proteases across neonatal intestine and development of liver disease in infants with alpha 1–antitrypsin deficiency. Lancet 1982; 1:1441-3.
68. Walker WA, Isselbacher KJ. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders. Gastroenterology 1974;67:531-50.
69. Lee EJ, Heiner DC. Allergy to cow milk—1985. Pediatr Rev 1986;7:195-203.
70. Robertson DM, Paganelli R, Dinwiddie R, Levinsky RJ. Milk antigen absorption in the preterm and term neonate. Arch Dis Child 1982;57:369-72.
71. Beach RC, Menzies IS, Clayden GS, Scopes JW. Gastrointestinal permeability changes in the preterm neonate. Arch Dis Child 1982;57:141-5.
72. Weaver LT, Laker MF, Nelson R. Intestinal permeability in the newborn. Arch Dis Child 1984;59:236-41.
73. Weaver LT, Laker MF, Nelson R, Lucas A. Milk feeding and changes in intestinal permeability and morphology in the newborn. J Pediatr Gastroenterol Nutr 1987;6:351-8.
74. Udall JN, Colony P, Fritze L, Pang K, Trier JS, Walker WA. Development of gastroinestinal mucosal barrier. II. The effect of natural versus artificial feeding on intestinal permeability to macromolecules. Pediatr Res 1981;15:245-9.
75. Kingham JGC, Whorwell PJ, Lochry CA. Small intestinal permeability. I. Effects of ischaemia and exposure to acetyl salicylate. Gut 1976;17:354-61.
76. Bloch KJ, Bloch DB, Stearns M, Walker WA. Intestinal uptake of macromolecules. VI. Uptake of protein antigen in vivo in normal rats and in rats infected with *Nippostrongylus brasiliensis* or subjected to mild systemic anaphylaxis. Gastroenterology 1979;77:1039-44.
77. Laker MF, Menzies IS. Increase in human intestinal permeability following ingestion of hypertonic solutions. J Physiol 1977;265:881-94.
78. Wheeler PG, Menzies IS, Creamer B. Effect of hyperosmolar stimuli and coeliac disease on the permeability of the human gastrointestinal tract. Clin Sci Mol Med 1978;54:495-501.
79. Wells CL, Maddaus MA, Simmons RL. Proposed mechanisms for the translocation of intestinal bacteria. Rev Infect Dis 1988;10:958-79.
80. Glode MP, Sutton A, Moxon ER, Robbins JB. Pathogenesis of neonatal *Escherichia coli* meningitis: induction of bacteremia and meningitis in infant rats fed *E. coli* K1. Infect Immun 1977;16:75-80.
81. Pluschke G, Mercer A, Kusecek B, Pohl A. Achtman M. Induction of bacteremia in newborn rats by *Escherichia coli* K1 is correlated with only certain O (lipopolysaccharide) antigen types. Infect Immun 1983;39:599-608.
82. Mizrahi A, Barlow O, Berdon W, Blanc WA, Silverman WA. Necrotizing enterocolitis in premature infants. J PEDIATR 1965;66:697-706.
83. Seidman EG, Hanson DG, Ely I, Udall JN, Walker WA. Regional colonic uptake of macromolecules: closed loop studies in rabbits at different ages [Abstract]. Gastroenterology 1986;90:1625.
84. Pitt J, Barlow B, Heird WC. Protection against experimental necrotizing enterocolitis by maternal milk. I. Role of milk leukocytes. Pediatr Res 1977;11:906-9.
85. Barlow B, Santulli TV, Heird WC, Pitt J, Blanc WA, Schullinger JN. An experimental study of acute neonatal enterocolitis: the importance of breast milk. J Pediatr Surg 1974;9:587-94.
86. Santulli TV, Schullinger JN, Heird WC, et al. Acute necrotizing enterocolits in infancy: a review of 64 cases. Pediatrics 1975;55:376-87.
87. Buckley RH, Dees SC. Correlation of milk precipitins with IgA deficiency. N Engl J Med 1969;281:465-9.
88. Cunningham-Rundles C. Failure of antigen exclusion. In: Brostoff J, Challacombe SJ, eds. Food allergy and intolerance. London: Baillière Tindall, 1987:223-36.
89. Ogra PL, Greene HL. Human milk and breast feeding: an update on the state of the art. Pediatr Res 1982;16:266-71.
90. Bauer CR, Morrison JC, Poole WK, et al. A decreased incidence of necrotizing enterocolitis after prenatal glucocorticoid therapy. Pediatrics 1984;73:682-8.
91. Lebenthal E, Leung YK. Feeding the premature and compromised infant: gastrointestinal considerations. Pediatr Clin North Am 1988;35:215-38.

92. Celano P, Jumawan J, Horowitz C, Lau H, Koldovsky O. Prenatal induction of sucrase activity in rat jejunum. Biochem J 1977;162:469-72.
93. Pang KY, Bresson JL, Walker WA. Development of the gastrointestinal mucosal barrier: evidence for structural differences in microvillus membranes from newborn and adult rabbits. Biochim Biophys Acta 1980;727:201-8.
94. Schwarz SM, Hostetler B, Ling S, Mone M, Watkins JB. Intestinal membrane lipid composition and fluidity during development in the rat. Am J Physiol 1985;248:G200-7.
95. Chu SHW, Walker WA. Development of the gastrointestinal mucosal barrier: changes in phospholipid head groups and fatty acid composition of intestinal microvillus membranes from newborn and adult rats. Pediatr Res 1988;23:439-42.
96. Neu J, Ozaki CK, Angelides KJ. Glucocorticoid-mediated alteration of fluidity of brush-border membrane in rat small intestine. Pediatr Res 1986;20:79-82.
97. Blum PM, Phelps DL, Ank BJ, Krantman HJ, Stiehm ER. Survival of oral human immune serum globulin in the gastrointestinal tract of low birth weight infants. Pediatr Res 1981;15;1256-60.
98. Mietens C, Keinhorst H, Hilpert H, Gerber H, Amster H, Pahud JJ. Treatment of infantile *E. coli* gastroenteritis with specific bovine anti–*E. coli* milk immunoglobulins. Eur J Pediatr 1979;132:239-52.
99. Tachet CO, Losonsky G, Link H, et al. Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic *Escherichia coli*. N Engl J Med 1988;318: 1240-3.
100. Eibl MM, Wolf HM, Furnkranz H, Rosenkranz A. Prevention of necrotizing enterocolitis in low-birth-weight infants by IgA-IgG feeding. N Engl J Med 1988;319:1-7.
101. Han VKM, Sayed H, Chance GW, Brabyn DG, Shaheed WA. An outbreak of *Clostridium difficile* necrotizing enterocolitis: a case for oral vancomycin therapy? Pediatrics 1983;71:935-41.
102. Egan EA, Mantilla G, Nelson RM, Eitzman DV. A prospective, controlled trial of oral vancomycin in the prevention of neonatal necrotizing enterocolitis. J PEDIATR 1976;89:467-70.
103. Boyle R, Nelson JS, Stonestreet BS, Peter G, Oh W. Alterations in stool flora resulting from oral kanamycin prophylaxis of necrotizing enterocolitis. J PEDIATR 1978;93:857-61.
104. Grylack LJ, Scanlon JW. Oral gentamicin therapy in the prevention of neonatal necrotizing enterocolitis. Am J Dis Child 1978;132:1192-4.
105. Rowley MP, Dahlenberg GW. Gentamicin in prophylaxis of neonatal necrotising enterocolitis [Letter]. Lancet 1978;2:532.
106. Ng PC, Dear PRF, Thomas DFM. Oral vancomycin in prevention of necrotizing enterocolitis. Arch Dis Child 1988; 63:1390-3.
107. Rotbart HA, Levin MJ. How contagious is necrotizing enterocolitis? Pediatr Infect Dis 1983;2:406-13.
108. Rousset S, Moscovici O, Lebon P, et al. Intestinal lesions containing coronavirus-like particles in neonatal necrotizing enterocolitis: an ultrastructural analysis. Pediatrics 1984; 73:218-24.
109. Resta S, Luby JP, Rosenfeld CR, Siegel JD. Isloation and propagation of a human enteric coronavirus. Science 1985;229:978-81.
110. McNeil PL, Ito S. Gastroinestinal cell plasma membrane wounding and resealing *in vivo*. Gastroenterology 1989; 96:1238-48.
111. Wilson R, Kanto WP Jr, McCarthy BJ, Burton A, Lewin P, Feldman RA. Short communication: age at onset of necrotizing enterocolitis—an epidemiologic analysis. Pediatr Res 1982;16:82-4.
112. Wilson R, Kanto WP Jr, McCarthy BJ, Feldman RA. Age at onset of necrotizing enterocolitis: risk factors in small infants. Am J Dis Child 1982;136:814-6.
113. Gaynes RP, Palmer S, Martone WJ, et al. The role of host factors in an outbreak of necrotizing enterocolitis. Am J Dis Child 1984;138:1118-20.
114. Yu VYH, Joseph R, Bajuk B, Orgill A, Astbury J. Perinatal risk factors for necrotizing enterocolitis. Arch Dis Child 1984;59:430-4.
115. DeCurtis M, Paone C, Vetrano G, Romano G, Paludetto R, Ciccimarra F. A case-control study of necrotizing enterocolitis occurring over 8 years in a neonatal intensive care unit. Eur J Pediatr 1987;146:398-400.
116. Wiswell TE, Hankins CT. Twins and triplets with necrotizing enterocolitis. Am J Dis Child 1988;142:1004-6.
117. Sibbons P, Spitz L, van Valzen D, Bullock GR. Relationship of birth weight to the pathogenesis of nectrotizing enterocolitis in the neonatal pig. Pediatr Pathol 1988;8:151-62.