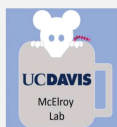


NEC and SIP: Intestinal disasters of the preterm infant

Steven McElroy, MD
Professor and Chief of Neonatology
Department of Pediatrics, UC Davis



UC DAVIS
HEALTH

CHILDREN'S
HOSPITAL



FANNP's National Neonatal Nurse Practitioner Symposium: Clinical Update and Review, 2025 ©



1

Disclosures

- Academic Advisory Board Member for NECSociety, Lactalogics, and Noveome
- Collaborations with Defensin Therapeutics (provides peptides)
- Funding through NIH/NIDDK
- All pictures were taken by me, obtained with parental consent, or freely available

2

Objectives

- What is NEC exactly?
- How do we think it occurs?
- What is new in our understanding?
- Why is our progress so slow?

3

Baby ES, born at 30 weeks



4

ES at 10 days of life



5

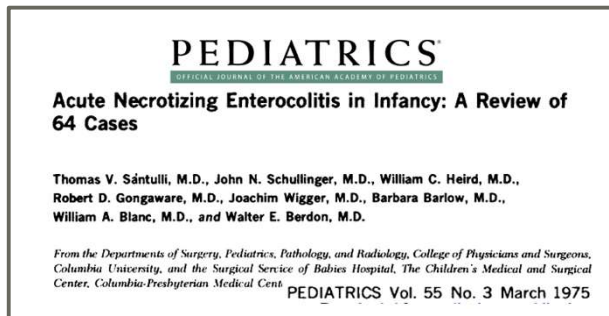
24 Hours Later



6

Necrotizing enterocolitis in the beginning

- First described in 1960's and 1970's
 - Mortality exceeded 70%
 - Initially described as idiopathic gastrointestinal perforations



7

Treating NEC In The Beginning

1970's Management

- Medical Management (10 day course)
 - NPO
 - Nasogastric suction
 - Systemic antibiotics
 - IV fluids
 - Monitoring with clinical signs and X-ray findings
- Surgical Management
 - Removal of necrotic/nonviable bowel
 - Re-anastomosis at a later time



8

Bell's staging

- First staging by Bell *et al* in '78
- Goal was to “effect uniformity in the therapeutic decisions which have been made in the management of these infants.”
- “An effort was made to assess severity of disease and provide graded intervention”

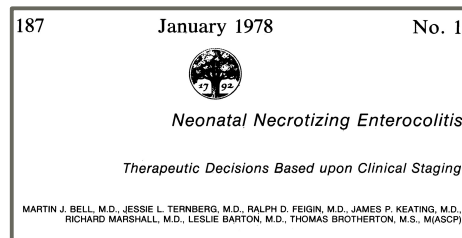


TABLE 1. NEC Staging System Based upon Historical, Clinical and Radiographic Data

STAGE I (Suspect)

- Any one or more historical factors producing perinatal stress.
- Systemic manifestations—temperature instability, lethargy, apnea, bradycardia.
- Gastrointestinal manifestations—poor feeding, increasing pre-gavage residuals, emesis (may be bilious or test positive for occult blood) mild abdominal distension, occult blood may be present in stool (no fissure).
- Abdominal radiographs show distension with mild ileus.

STAGE II (Definite)

- Any one or more historical factors.
- Above signs and symptoms plus persistent occult or gross gastrointestinal bleeding; marked abdominal distension.
- Abdominal radiographs show significant intestinal distension with ileus; small bowel separation (edema in bowel wall or peritoneal fluid), unchanging or persistent “rigid” bowel loops, pneumatosis intestinalis, portal vein gas.

STAGE III (Advanced)

- Any one or more historical factors.
- Above signs and symptoms plus deterioration of vital signs, evidence of septic shock or marked gastrointestinal hemorrhage.
- Abdominal radiographs may show pneumoperitoneum in addition to others listed in II c.

9

Necrotizing enterocolitis today

- An acute inflammatory disease process of the bowel
- Primarily a disease of premature infants
 - 5-7% in US less than 1500g birth weight
- Mortality rate of 30% and can account for 10% of deaths in the NICU



10

Onset of NEC is age-dependent

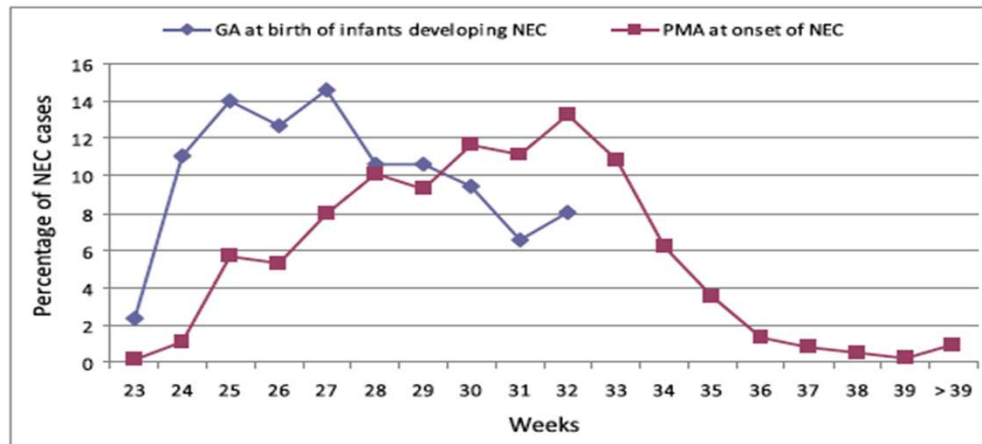


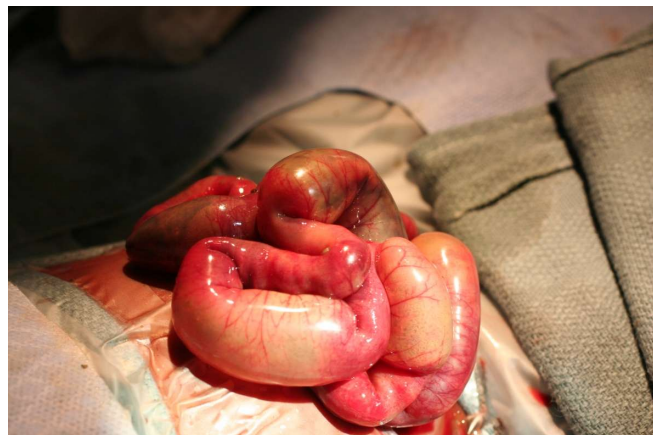
FIGURE 2
Distribution of cases of NEC according to GA and postmenstrual age (PMA) in the study cohort.

Yee, *Pediatrics* 2012

11

The pathology of necrotizing enterocolitis

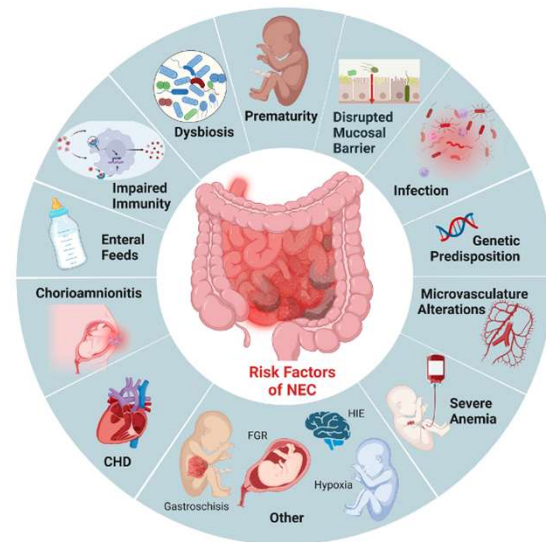
- Predominant lesion is necrosis
 - Coagulative: Blood engorged/venous obstruction
 - Ischemic: No blood flow/arterial
- Lesions can be continuous or discontinuous



12

Who is at risk for NEC?

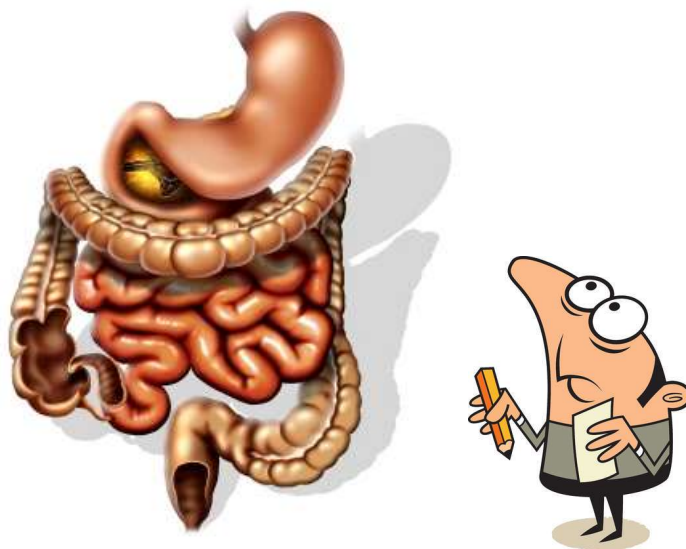
- NEC is not a single entity with a linear pathophysiology
- Instead, there are many risk factors
- And likely multiple pathways to incite the inflammatory spiral that ends in a common endpoint



Bautista *et al*, *Front Pediat* 2023

13

The intestinal tract is the largest part of our bodies that interact with the external environment.



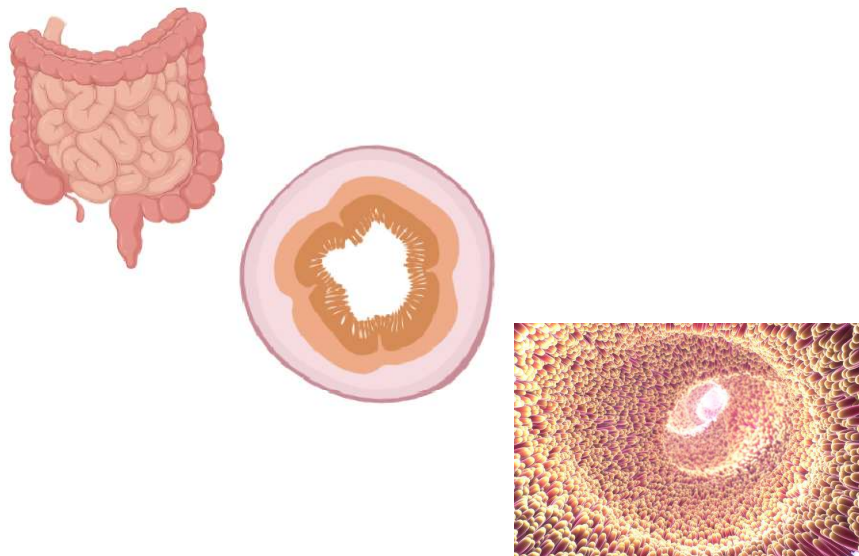
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Relative surface areas of human tissue.



15

The gut has so much surface area due to folding



16

All that separates us from the “outside” is a single epithelial layer of cells

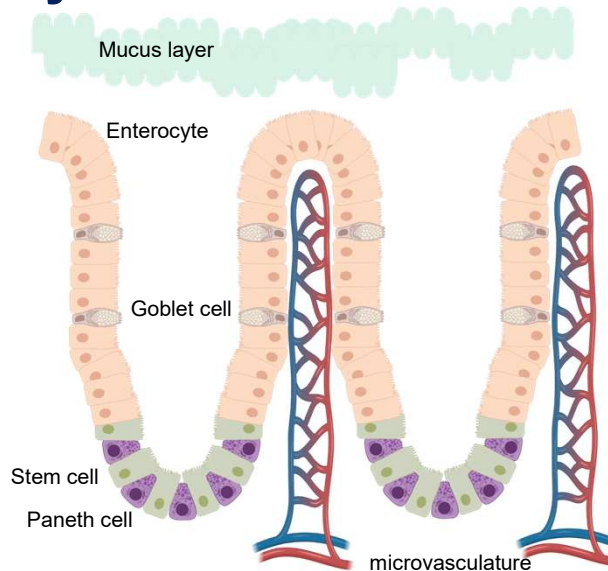
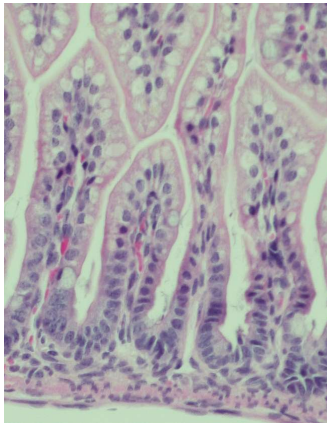


Image Source: McElroy Lab

17

- Life induces bacterial proliferation
- We have 1.5-10 times the bacteria in our intestine than we have cells in our body

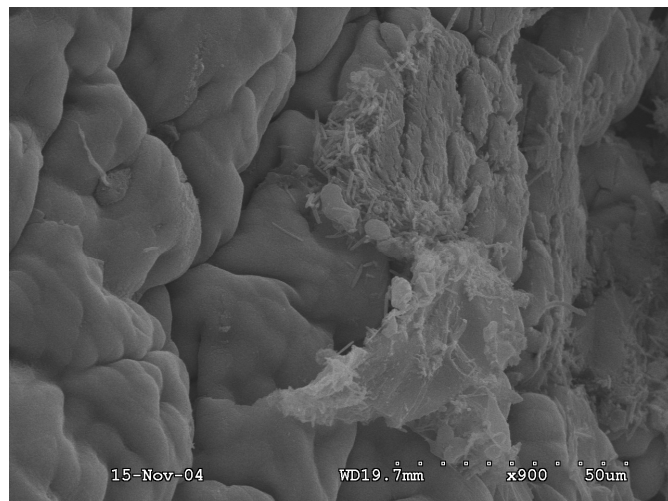
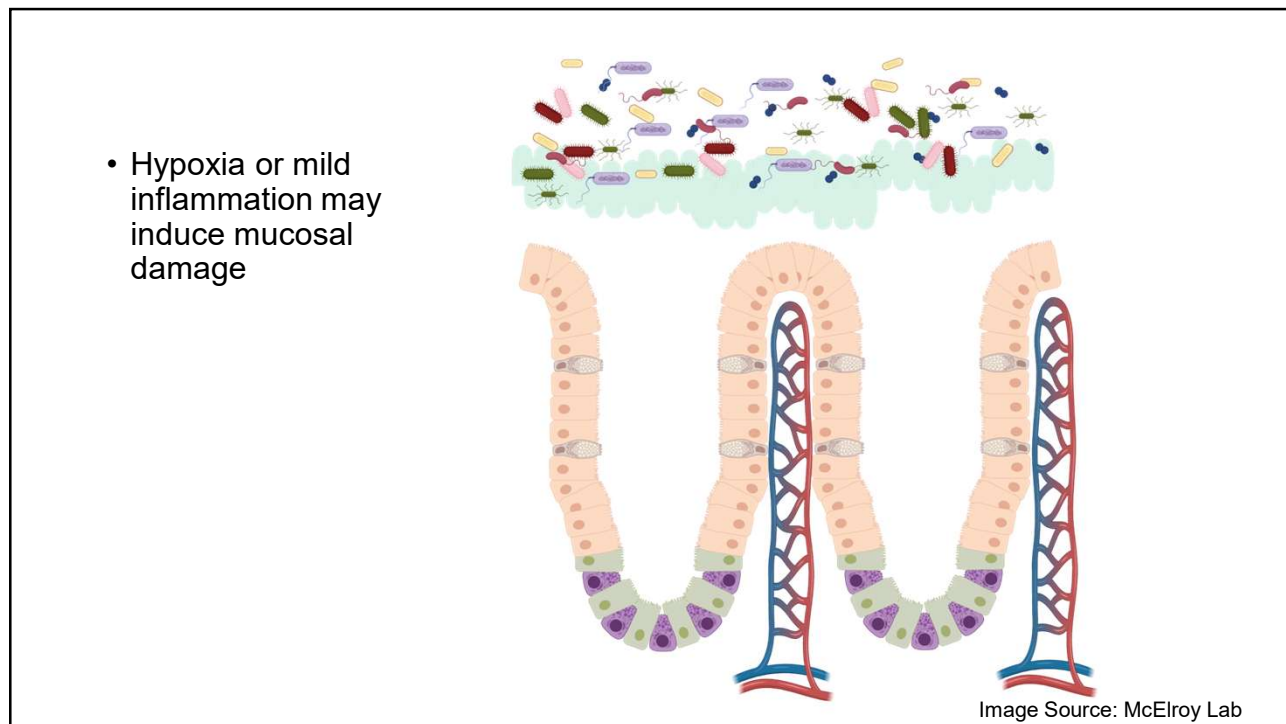


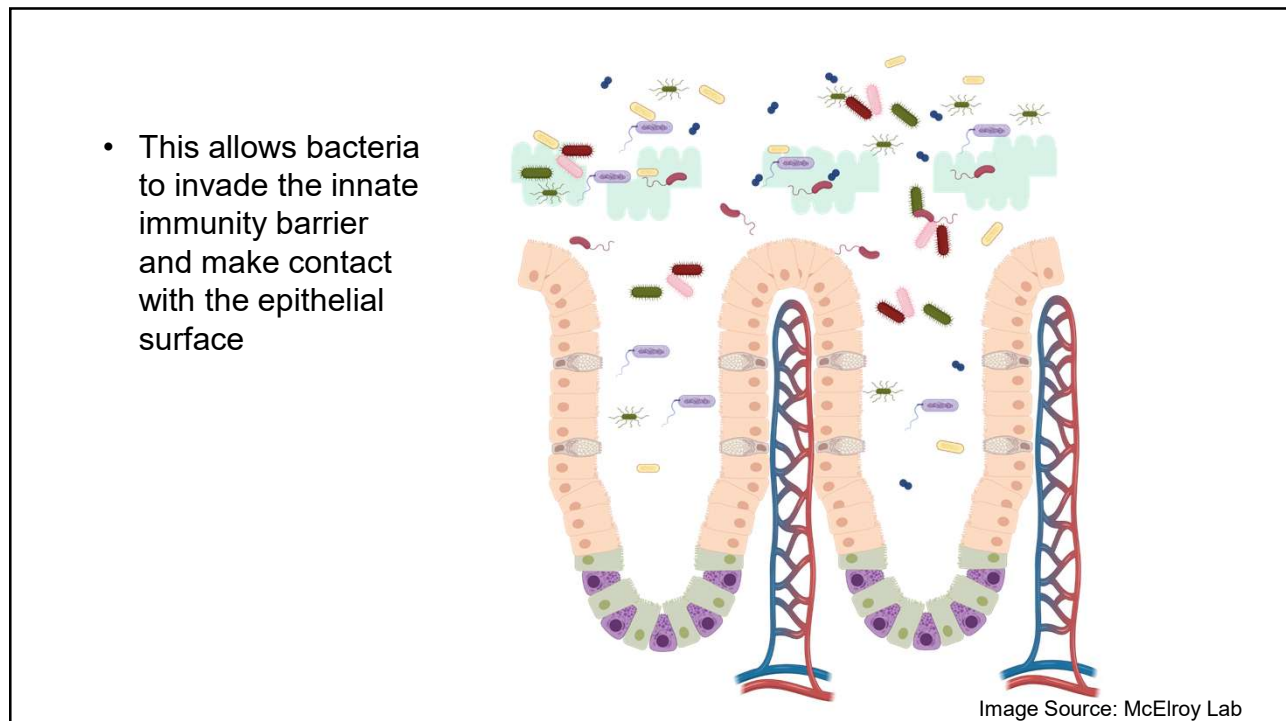
Image Source: Thaddeus S. Stappenbeck, Washington University School of Medicine

Gilbert J *et al. Nat Med.* 24, 392-400. 2018.

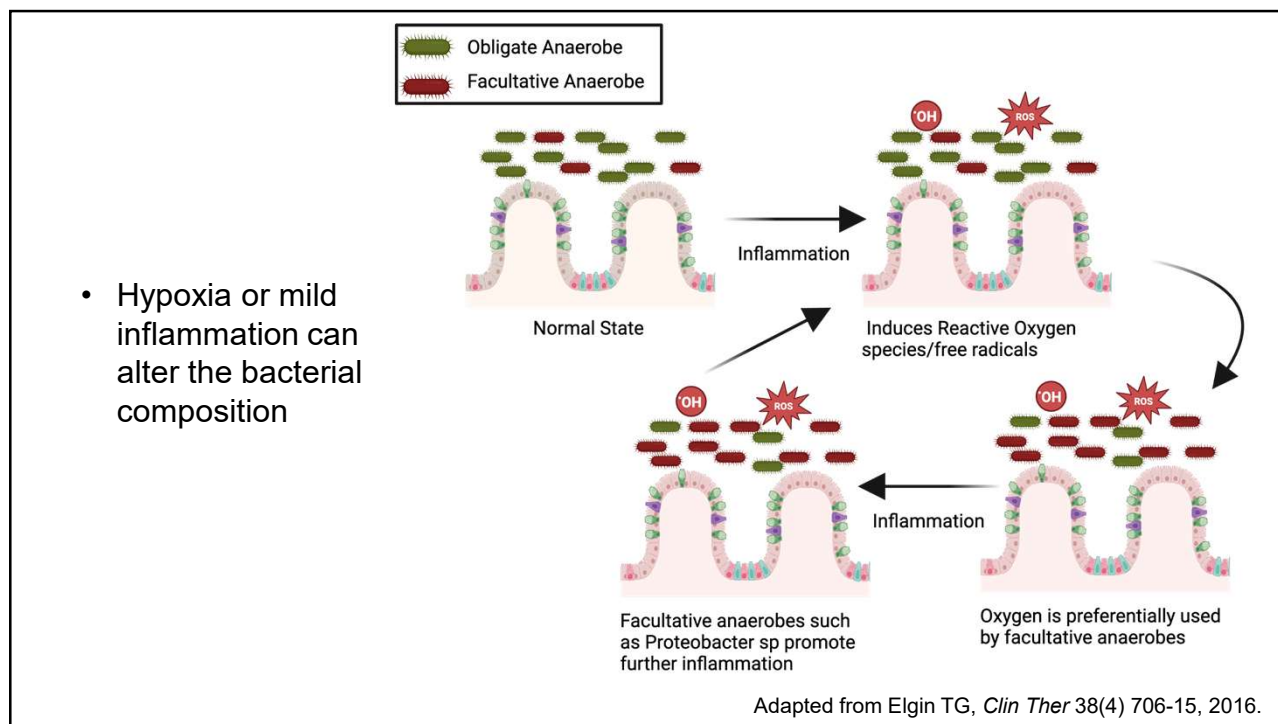
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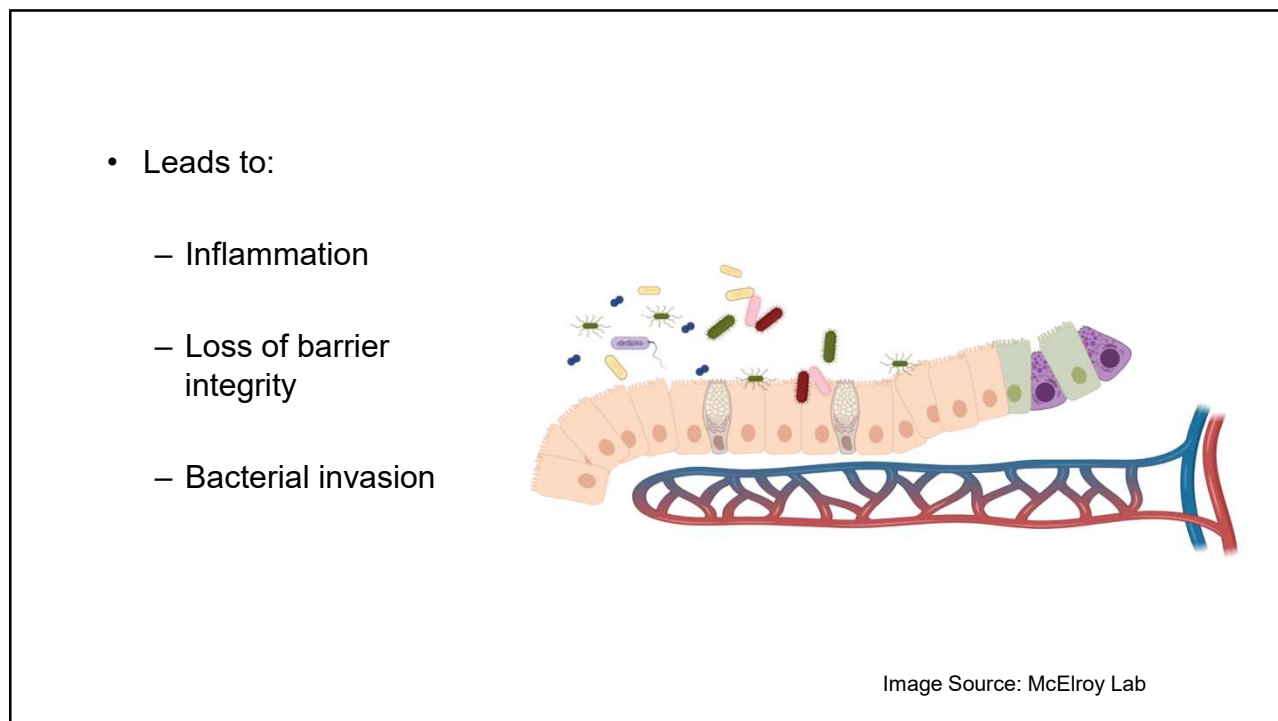
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22

Two ways that the epithelium responds

- Cellular signals from
 - Toll-like Receptors
 - Nucleotide-binding Oligomerization Domain-like Receptors
 - Danger-associated Molecular Pattern Receptors

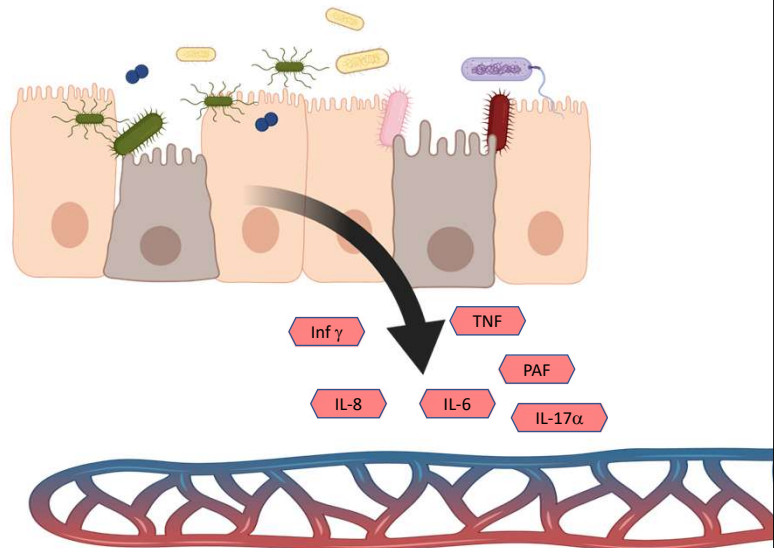


Image Source: McElroy Lab

23

Two ways that the epithelium responds

- Induction of Paneth cell disruption

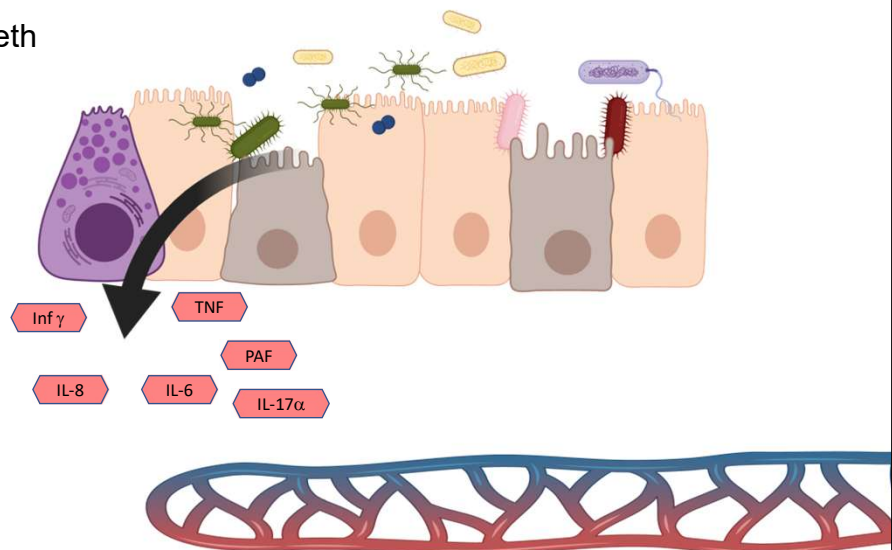
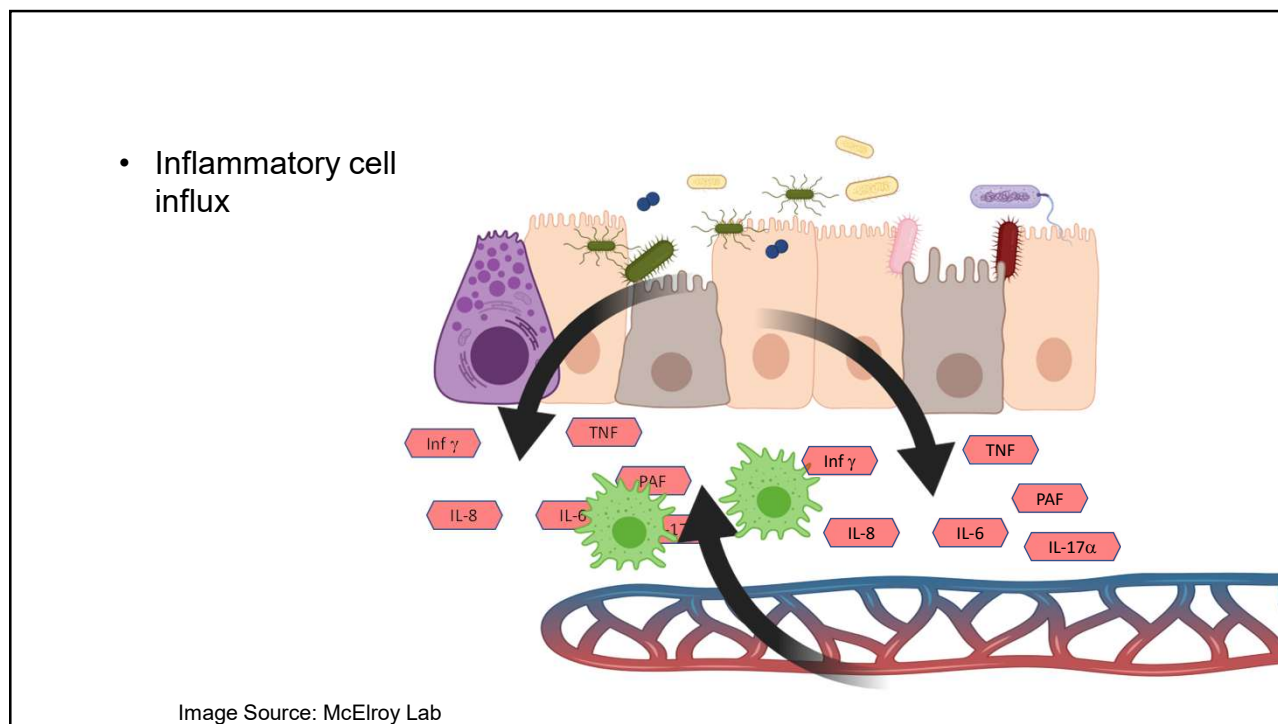
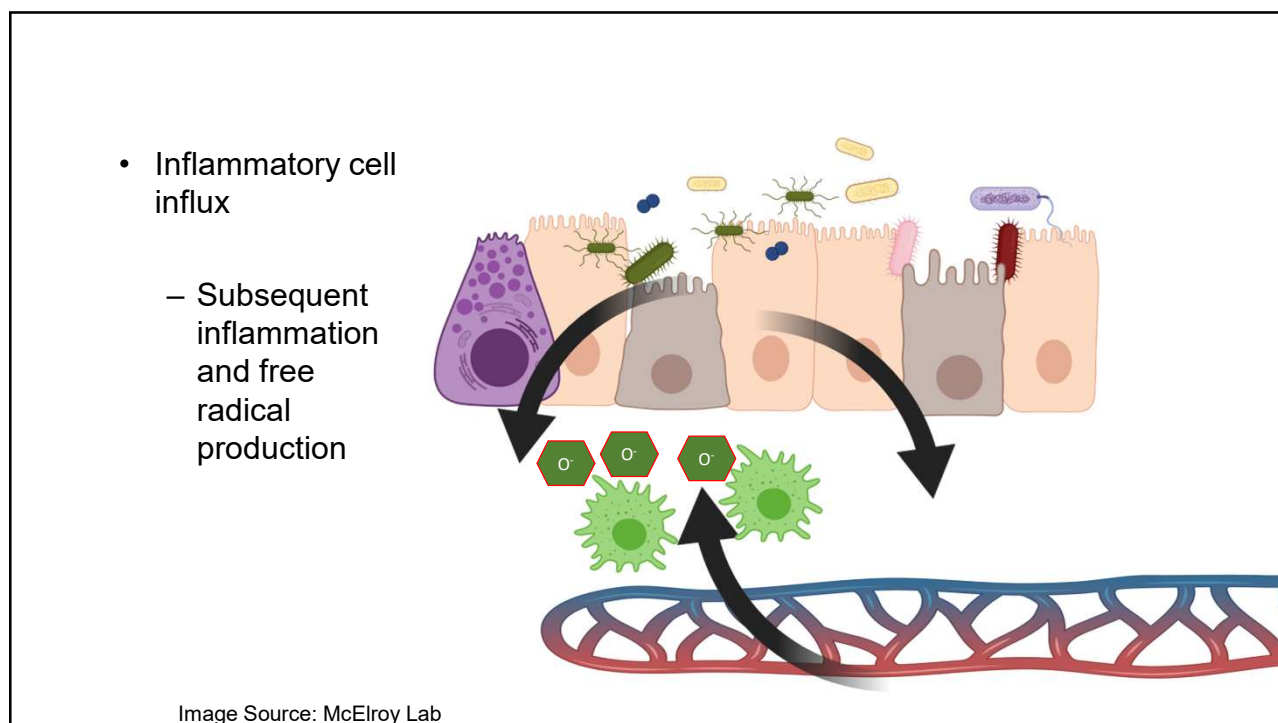


Image Source: McElroy Lab

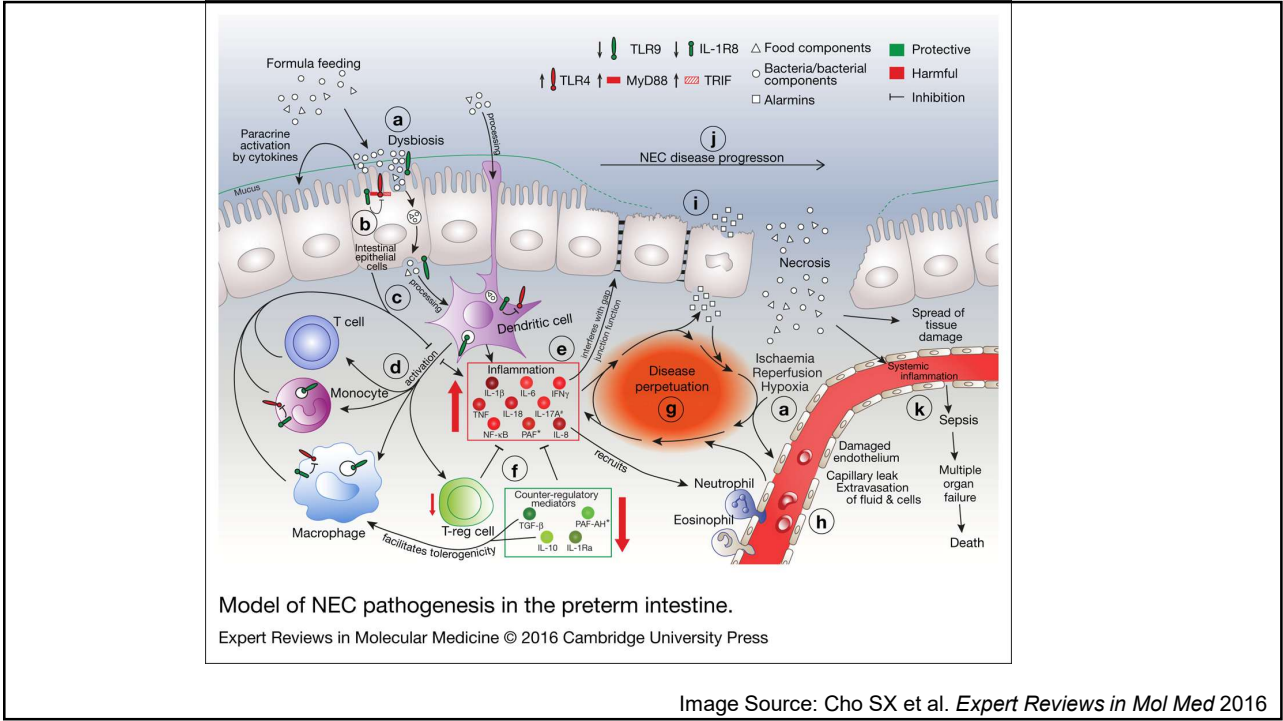
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26



27

Pneumatosis intestinalis

Portal Gas

Pneumatosis

Photo by SJ McElroy

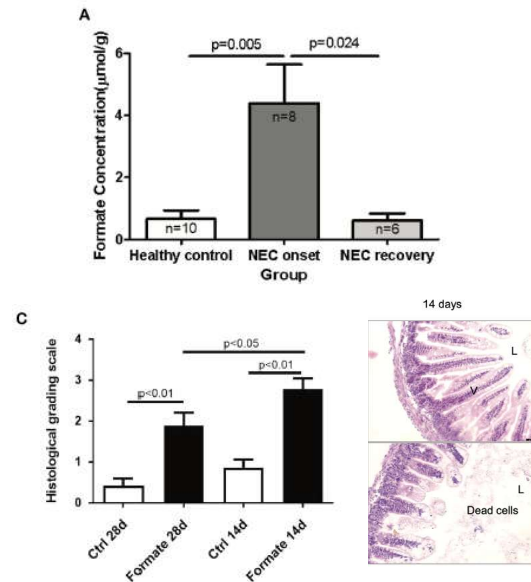
Histology from Cornell University Medical College

Radiograph and bowel picture with permission by SJ McElroy

28

Pneumatosis intestinalis

- Metabolomic analysis of 245 infants with NEC showed an association with increases in the short chain fatty acid formate in stool samples
- *Enterobacter cloacae* and *Klebsiella pneumoniae* both increase formate production
- Mice exposed to formate in the intestinal lumen developed epithelial injury and barrier dysfunction



Casaburi G et al, *Frontiers*, Aug 2022

29

But so far we have only talked about...



30

But NEC does happen at term...

- 10-20% of neonates with NEC are term
- Important to note: Term infants have essentially a fully mature intestine
- These infants have associated co-morbidities
 - Intrauterine growth restriction
 - Sepsis
 - Asphyxia
 - Neonatal abstinence syndrome
 - **Congenital Heart Disease**



31

Where does our information come from?

- Three large cohort studies
 - Christensen and Lambert (2013)
 - 11,596 infants from Intermountain Health from 2001-2011
 - Li (2017)
 - 70,326 from Children's Hospital of Chongqing from 1996-2015
 - Overman (2019)
 - 170 infants from CS Mott Children's Hospital from 2003-2012



32

Facts about term NEC

- Typically present at 6-22 days of life
 - Later onset seems to be more consistent with cardiac disease
- Bloody stools and radiologic changes are most often the first signs
- Most commonly have colon as site of NEC
 - Small intestine is the site of premie NEC



33

NEC and Congenital Heart Disease

- Risk factors:
 - Prematurity
 - Overfeeding (esp with formula)
 - Hypoplastic Left Heart Syndrome (may be as high as 8% develop NEC)
 - Truncus arteriosus
- Associated mortality
 - Premies: ~30%
 - Non-cyanotic CHD: 39%
 - Cyanotic CHD: 57%
 - Preterm with CHD: 43%



Lipman "One Portrait of One Man" Weisman Museum of Art

34

Term infants with CHD and NEC have abnormal blood flow patterns

- Persistent diastolic reversal in the abdominal aorta is associated with NEC in infants with CHD

Carlo Pediatrics 2007

- Study looked at 18 infants with CHD and NEC compared to 20 infants with CHD alone (matched diagnosis and age)

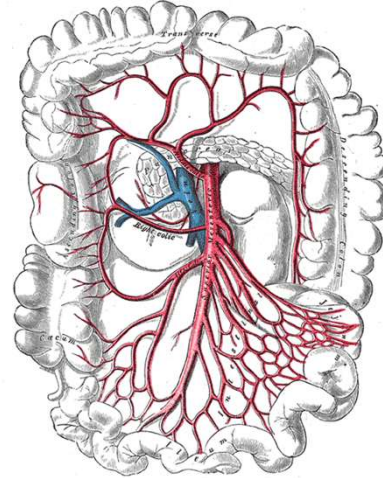


Image Source: Gray's Anatomy

35

This reduced blood flow may predispose to secondary injury

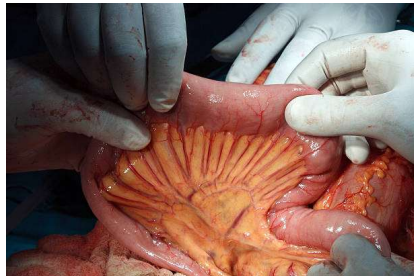
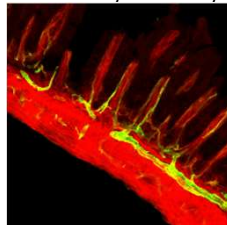
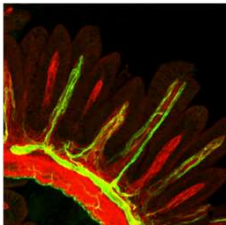
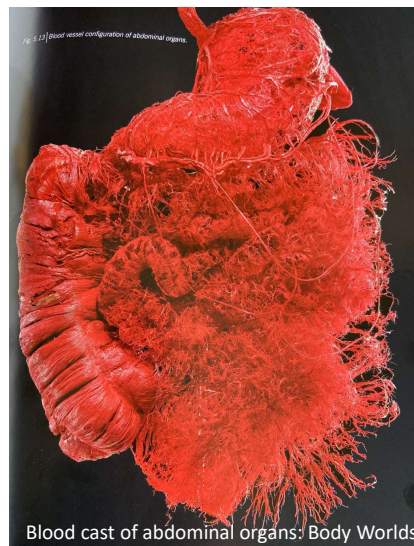


Photo by SJ McElroy



Berger JN, et al, *J Ped Surg*, 2019



36

Spontaneous or Focal Intestinal Perforation

- Process that is often confused with or hard to distinguish from NEC
- Realized to be a different entity than NEC in the late 80's
- Case report of 6 infants born 22-30 weeks with localized intestinal perforation unassociated with NEC or other GI anomaly

The JOURNAL
of PEDIATRICS

Spontaneous focal gastrointestinal perforation in very low birth weight infants

Judy L. Aschner, MD, Karl S. Deluga, MD, Leon A. Metlay, MD, Robert W. Emmens, MD, and Karen D. Hendricks-Munoz, MD

From the Department of Pediatrics, Division of Neonatology, and the Departments of Pathology and Surgery, University of Rochester Medical Center, Rochester, New York, and the Department of Neonatology, Providence Hospital, Southfield, Michigan

Aschner et al. *J Pediatr*. 1988 Aug

37

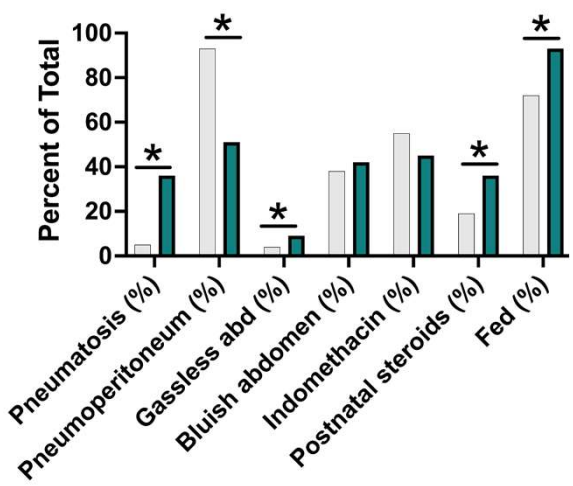
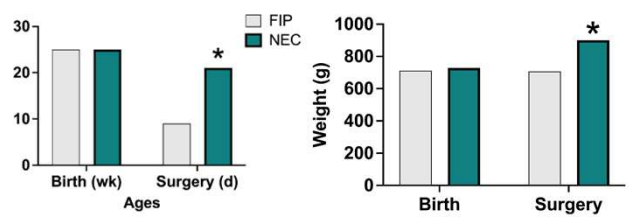
Spontaneous or Focal Intestinal Perforation

- Unclear mechanism/pathophysiology
- Thought to be due to ischemia possibly in combination with bowel wall thinning
 - Vascular malformations?
 - Maternal factors (pre-eclampsia, IUGR, antenatal medications)?
 - Early postnatal steroids especially with NSAID use?
 - Meconium obstruction?
- No animal models and limited clinical associative data

38

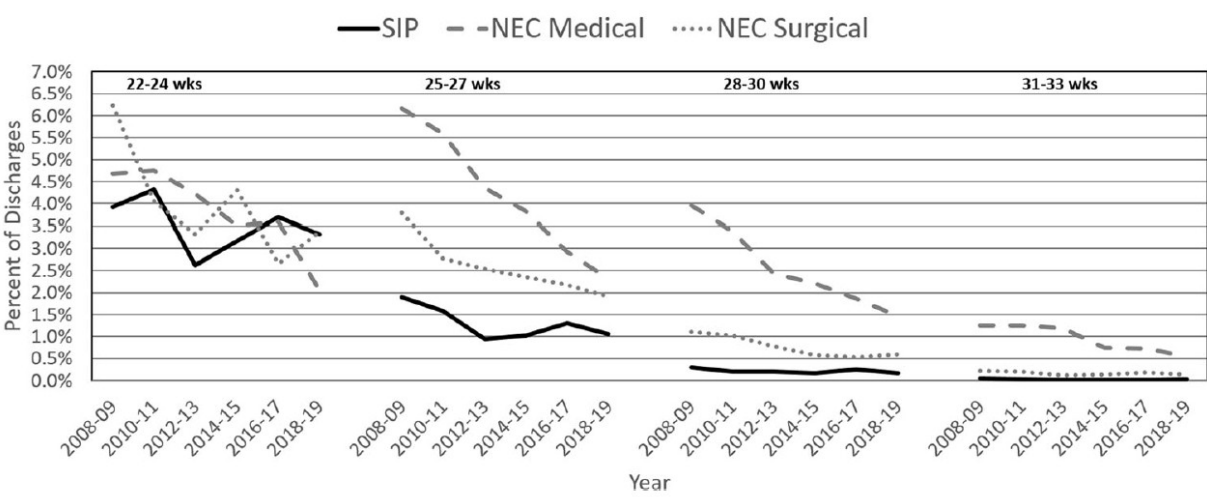
Spontaneous or Focal Intestinal Perforation

- Data from the NEST trial
 - Comparing laparotomy vs drain
 - Large randomized, multi-center NRN trial of 310 ELBW infants



Data generated from Rausch et al. Clin Perinatol. 2022

Spontaneous or Focal Intestinal Perforation



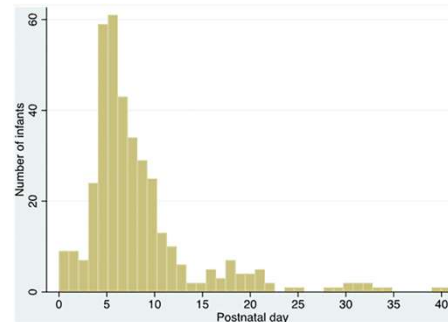
Swanson JR et al. J Perinatol. 2022

Spontaneous or Focal Intestinal Perforation

Risk Factors and Epidemiology of Spontaneous Intestinal Perforation Among Infants Born at 22–24 Weeks' Gestational Age

Pavan V Thakkar^{1,*}, Kent F Sutton², Chloe-Ann B Detwiler^{1,*}, Julia G Henegar^{1,*}, Jai R Narayan^{1,*}, Melanie Perez-Romero^{1,*}, Ciara M Strausser^{1,*}, Reese H Clark³, Daniel K Benjamin Jr^{1,4}, Kanecia O Zimmerman^{1,4}, Ronald N Goldberg⁴, Noelle Younge⁴, David Tanaka⁴, P Brian Smith^{1,4}, Rachel G Greenberg^{1,4}, Ryan Kilpatrick^{1,4}

- Observational cohort study
- 446 NICUs, 9712 infants
- Median GA 24 weeks and weight of 620g
 - 8% were 22 weeks
- SIP incidence
 - 6.1% at 22
 - 4.6% at 23
 - 3.3% at 24



41

Meconium-related obstruction?



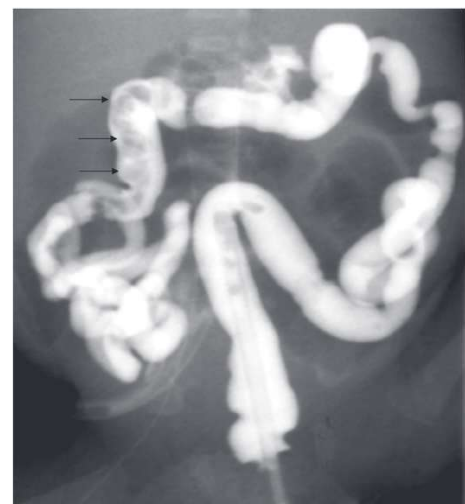
Pediatrics International (2011) **53**, 887–891

Meconium-related ileus in extremely low-birthweight neonates: Etiological considerations from histology and radiology

Akio Kubota,¹ Jun Shiraishi,² Hisayoshi Kawahara,¹ Hiroomi Okuyama,¹ Akihiro Yoneda,¹ Hiroshi Nakai,¹ Keigo Nara,¹ Hiroyuki Kitajima,² Masanori Fujimura,² Yuko Kuwae¹ and Masahiro Nakayama¹

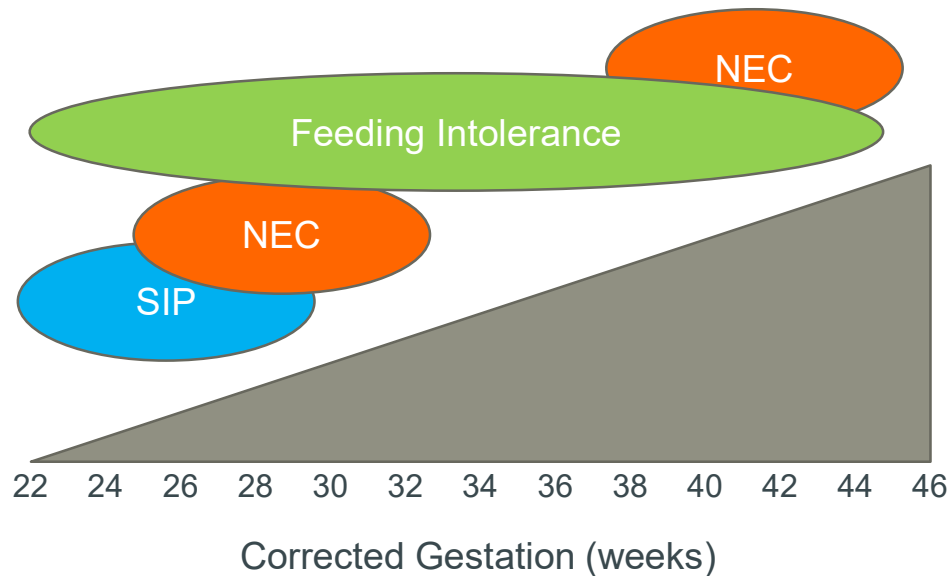
Departments of ¹Pediatric Surgery, ²Neonatology and ³Pathology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

- Recognized in Japan since the 90's
- Especially prevalent in 22-23 week infants
- Increasing awareness in US
- Treatment?



42

Disorders of the GI tract in the NICU



43

ONLY Known and Universally Accepted Preventative Measure Is Human Breast Milk

- Lancet article by Lucas and Cole in 1990 showed a decreased number of NEC cases in prospective study of 926 infants
 - Infants fed breastmilk developed **6-10 times less NEC** than infants fed formula alone and **3 times less** than infants fed breast milk + formula
- Several groups have demonstrated a decrease in experimental NEC when feeding rat pups maternal milk instead of formula

44

- This stinks



45

- Do you have anything new?



46

Recent hot topics surrounding NEC



47

- What to eat
- Probiotics
- A lesson from blood
- Antibiotics



48

Human milk reduces infant infections

- In a study looking at 10,947 breastfed infants in Ghana from 2003-2004, 16% of neonatal deaths could be saved if all infants were breastfed from day 1

Edmond KM. Pediatrics. 2006

- NICU patients <1500g at birth fed human milk had ½ the infections of infants fed formula

Hylander MA. Pediatrics. 1998

49

Human milk reduces NEC

- Prospective study by Lucas and Cole of 926 infants
 - Infants fed breastmilk developed **6-10 times less NEC** than infants fed formula alone and **3 times** less than infants fed breast milk + formula

Lucas A. Lancet 1991

- Schanler also found decreases in NEC of infants fed fortified human milk compared to those fed preterm formula

Schanler RJ, et al. Pediatrics 1999

- Multiple groups have demonstrated a decrease in experimental NEC in preclinical models comparing maternal milk vs formula

50

Donor milk also reduces NEC

- Double-blind, randomized trial
 - 363 infants (181 donor milk, 180 formula)
 - NEC stage 2 or higher was 6.6% in formula, 1.7 in donor milk p=0.02

JAMA | Original Investigation

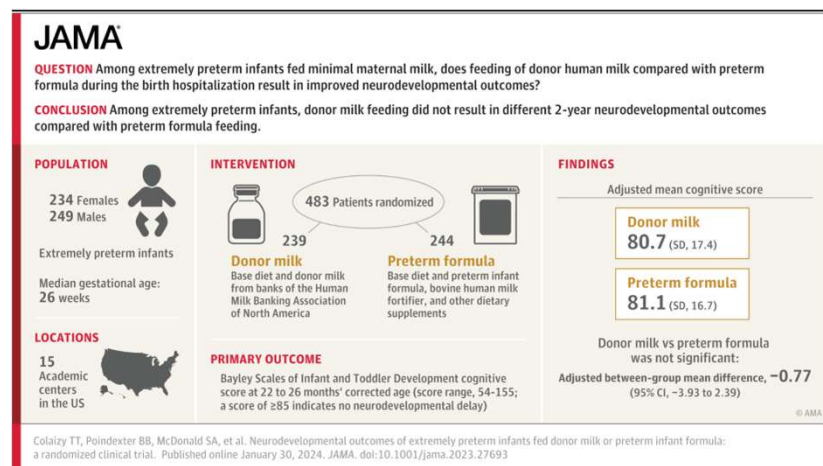
Effect of Supplemental Donor Human Milk Compared With Preterm Formula on Neurodevelopment of Very Low-Birth-Weight Infants at 18 Months A Randomized Clinical Trial

Deborah L. O'Connor, PhD, RD; Sharyn Gibbins, PhD, RN; Alex Kiss, PhD; Nicole Bando, MSc; Joan Brennan-Donnan, MSc, RD; Eugene Ng, MD; Douglas M. Campbell, MD, MSc; Simone Vaz, MD; Christoph Fusch, MD, PhD; Elizabeth Asztalos, MD, MSc; Paige Church, MD; Edmond Kelly, MD; Linh Ly, MD; Alan Daneman, MD; Sharon Unger, MD; for the GTA DoMINO Feeding Group

51

Donor milk neurodevelopmental outcomes are not inferior to formula (base diet)

- Double-blind, randomized trial
 - Infants < 29 w or 1000g BW
 - 206 infants (239 donor milk, 244 formula)
 - No significant difference in ND outcomes
 - NEC rate was reduced by ½ in donor milk group



52

Not all milk is the same



| Species of Mammal | Weight at birth | Weight at 3 months | Weight at 8 months | Weight at 1 year | Milk Fat Content |
|-------------------|-----------------|--------------------|--------------------|------------------|------------------|
| Human | 7.5 lbs | 14 lbs | 19 lbs | 21 lbs | 4.2% |
| Holstein Cow | 57-91 lbs | 250 lbs | 480 lbs | 700 lbs | 3.6% |
| Polar Bear | 1-1.5 lbs | 25 lbs | 99 lbs | | 33% |
| Orca Whale | 300-400 lbs | | | 1400 lbs | 48% |

53

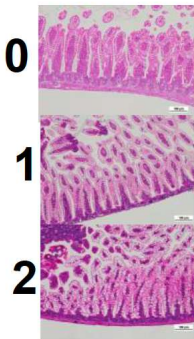
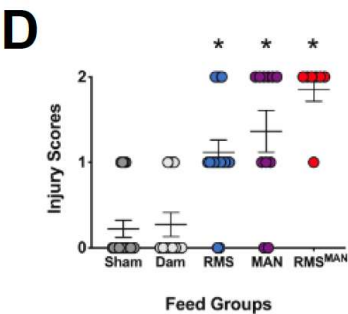
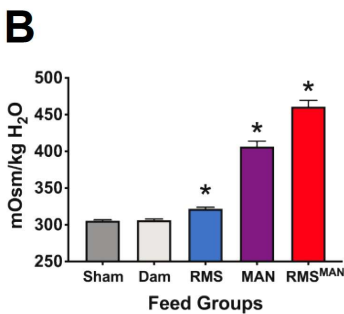
But is formula bad?

- While it is clear human milk *reduces* the incidence of NEC it doesn't *eliminate* it
- Most studies that use human milk diets including human fortifiers still have NEC rates ~3%
- Most animal models utilize bovine-based formula to induce NEC, however in none of these is the formula sufficient alone

54

But is formula bad?

- While high osmolality formula can cause intestinal injury, injury is osmolality-dependent and requires levels higher than is found in modern NICUs



Lueschow SR et al, *Nutrients*, 2020

55

But is formula bad?

Reuters World Business Markets Sustainability Legal Breakingviews Technology Investigations

Healthcare & Pharmaceuticals | Product Liability | Corporate Structure | Medtech | Lawsuits

Abbott must pay \$495 million in premature infant formula trial, jury finds

By Brendan Pierson and Dietrich Knauth
July 26, 2024 5:52 PM PDT · Updated 4 days ago

Abbott Laboratories logo is displayed on a screen at the New York Stock Exchange (NYSE) in New York City, U.S., October 18, 2021.
REUTERS/Brendan McDermid/File Photo [Purchase Licensing Rights](#)

56

But is formula bad?



Oct 3, 2024

FDA, CDC, NIH Consensus Statement on Recent Advisory Council Report on Premature Infants and Necrotizing Enterocolitis

57

But is formula bad?



Oct 3, 2024

- For infants where the supply of human milk is insufficient, these formulas are part of the standard of care for premature infants
- There are two key points about feeding practices and NEC: 1) There is no conclusive evidence that preterm infant formula causes NEC; and 2) there is strong evidence that human milk is protective against NEC
- All infants should be fed as soon as is medically feasible through whatever appropriate nutritious food source is available.

58

EBM is best! But not evidence that formula is inherently bad!

- Human milk is recognized as the best source of nutrition
- Studies have not explained why human milk reduces NEC
- Studies have not explained why infants fed exclusive BM develop NEC (~3%)
- Thus, claims that formula causes NEC are only speculative



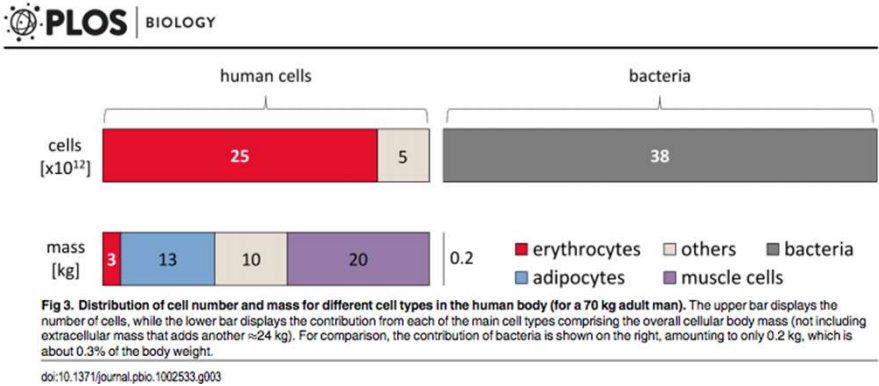
59

- What to eat
- Probiotics
- A lesson from blood
- Antibiotics



60

How much bacteria is there?



Sender R *et al. PLoS Biol* 14(8); e1002533, 2016.

61

How much of you is really you?

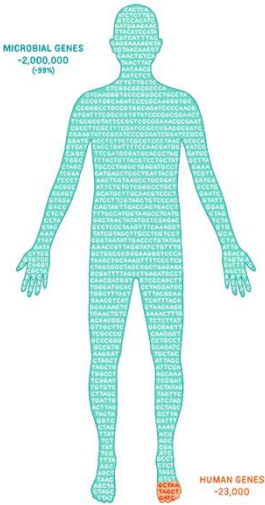
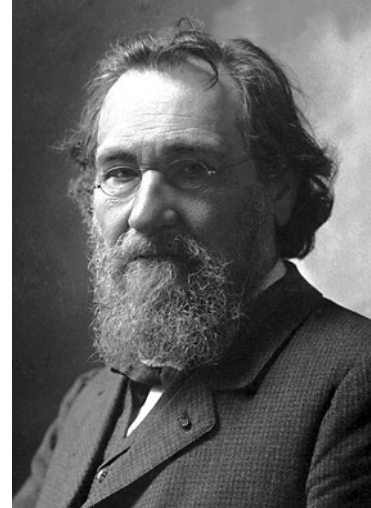


Image Source: Gabby D'Allesandro/American Museum of Natural History

62

What is a Probiotic?

- The idea that intestinal bacteria can affect our health was first proposed in 1907 Dr. Elie Metchnikoff, a Russian zoologist and microbiologist
- Proposed that consuming fermented milk was health promoting by preventing bacterial growth
- Live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance



63

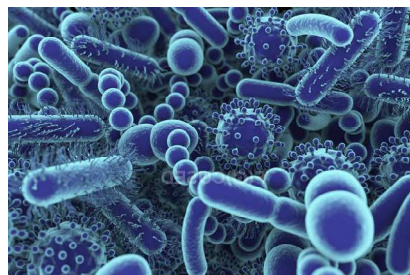
How about in humans?

- Probiotics have now been studied in over 10,000 preterm infants
- Most common probiotics used include *Bifidobacterium*, *Lactobacillus*, or a combination



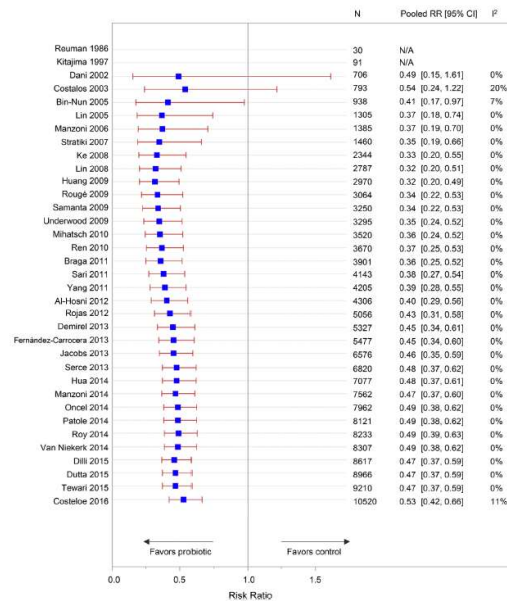
Probiotics and necrotizing enterocolitis

Ravi Mangal Patel, MD, MSc^{a,*}, Mark A. Underwood, MD, MAS^b



64

Human preterm trials of probiotics



Patel and Underwood, *Semin Pediatr Surg* 2018 Feb;27(1):39-46.

65

Example

ORIGINAL ARTICLES

www.jpeds.com • THE JOURNAL OF PEDIATRICS

***Bifidobacterium longum* subsp. *infantis* EVC001 Administration Is Associated with a Significant Reduction in the Incidence of Necrotizing Enterocolitis in Very Low Birth Weight Infants**

Joseph Tobias, MD, MA¹, Amy Olyaei, BS², Bryan Laraway, MS³, Brian K. Jordan, MD, PhD², Stephanie L. Dickinson, MS⁴, Lilian Golzarri-Arroyo, MS⁴, Elizabeth Fialkowski, MD¹, Arthur Owora, MPH, PhD⁴, and Brian Scottoline, MD, PhD²

- Single center retrospective cohort study from 2014-2020
- 301 infants (control) compared to 182 infants (EVC001)
 - <1500g birth weight

66

Example

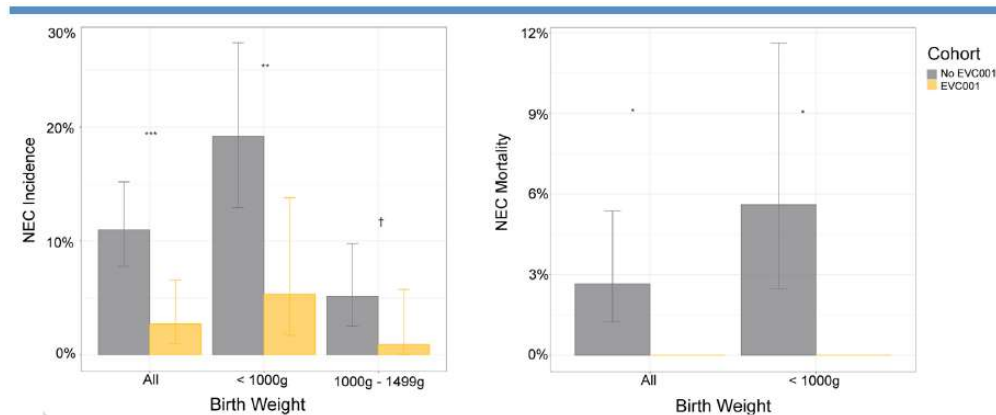


Figure 3. A, NEC incidence by birth weight and cohort. Error bars show 95% CIs around the estimates. *** $P < .001$; ** $P < .01$; $^{\dagger}P < .1$, Fisher exact test. B, NEC-related mortality rates by birth weight and cohort. Error bars show 95% CIs around the estimates. * $P < .05$, Fisher exact test.

67

Position paper by the AAP Committee on Fetus and Newborn

PEDIATRICS®

OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

American Academy
of Pediatrics



DEDICATED TO THE HEALTH OF ALL CHILDREN®

September 16, 2021

- Current evidence does not support the routine, universal administration of probiotics to preterm infants, particularly those with a birth weight of <1000 g.

68

Recent statements by the FDA



Information on Probiotic Safety

The FDA cautions that microorganisms contained in probiotics have been reported in the medical literature as causing bacteremia or fungemia, sometimes with a severe clinical course, in very preterm or very low birthweight (VLBW) infants.¹⁻⁴

Moreover, the American Academy of Pediatrics states “Given the lack of FDA-regulated pharmaceutical-grade products in the United States, conflicting data on safety and efficacy, and potential for harm in a highly vulnerable population, current evidence does not support the routine, universal administration of probiotics to preterm infants, particularly those with a birth weight of <1000 g.”⁵

The FDA is also reminding healthcare providers that FDA has not approved any probiotic product for use as a drug or biological product in infants. The FDA is aware that some unapproved, unlicensed probiotics are nonetheless sold for use to treat or prevent a disease or condition in infants, including to reduce the

69



70

Not all strains of probiotic are the same...





71

Not all strains of probiotic are the same...



72

Not all strains of probiotic are the same...



73

So why are these trials not consistent?

Updated Meta-analysis of Probiotics for Preventing Necrotizing Enterocolitis in Preterm Neonates

Girish Deshpande, Shripada Rao, Sanjay Patole and Max Bulsara
Pediatrics 2010;125:921-930; originally published online Apr 19, 2010;
DOI: 10.1542/peds.2009-1301

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Different probiotics
used in probiotic
trials

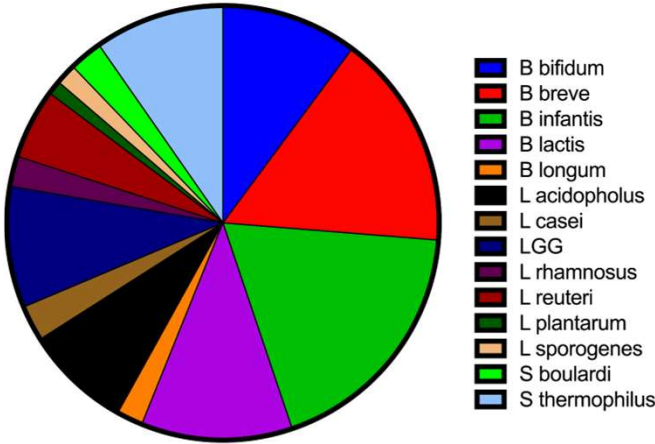
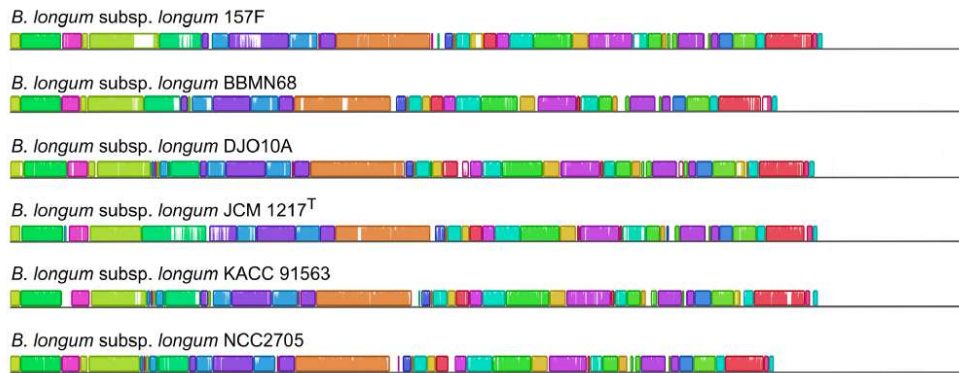


Image Source: McElroy Lab

74

Not all strains of probiotic are the same...

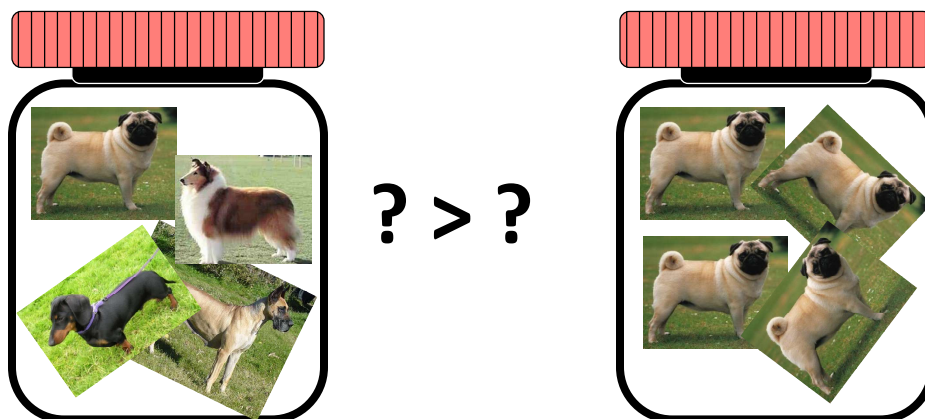


- Genetic variance from different strains of *Bifidobacterium longum* subspecies *longum*

Chaplin et al, *PLoS One*. 2015 Aug 14;10(8):e0135658.

75

So, are combinations of probiotics better?



76

So how about the IBT Connection Trial?

- Recently completed randomized, double blind, parallel-group, placebo-controlled clinical trial
- First dose administered ≤ 48 hours after birth and continued daily until the subject reaches 34 weeks + 6 days Post-Menstrual Age
- To evaluate the efficacy of IBP-9414 vs. placebo on the prevention of NEC and on sustained feeding tolerance
- Birth weight of 500-1500g, and gestational age of 23.0 – 32.0

77

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- To evaluate the efficacy of IBP-9414 vs. placebo on the prevention of NEC and on sustained feeding tolerance
- Birth weight of 500-1500g, and gestational age of 23.0 – 32.0
- Results are not yet published but IBT announced that there were no effects on NEC, but there was a significant decrease in all-cause mortality.

78

- What to eat
- Probiotics
- A lesson from blood
- Antibiotics



79

Transfusions and NEC

- Christensen (2010)
 - Retrospective study (112 infants) at 18 hospitals over 8 years
 - Odds of receiving a transfusion 48 hours before NEC was significantly higher among infants who subsequently developed NEC ($p < 0.001$)
 - Infants developing NEC after PRBC also had significantly higher feed volumes before and during transfusion
- Josephson (2010)
 - Retrospective study (93 infants) at two institutions
 - 38% of patients who developed NEC had received a transfusion within 48 hours prior to diagnosis
 - Infants who received transfusions were born at earlier ages, had greater intensive care needs, and had longer hospital stays

80

Transfusions and NEC

- Infants who had transfusions associated with development of NEC also have
 - Lower gestation
 - Lower birth weight
 - Higher risk of PDA
 - Higher risk of being ventilated at the time of diagnosis
 - Higher rates of mortality

81

Transfusions and NEC

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 - Lower gestation
 - Lower birth weight
 - Higher risk of PDA
 - Higher risk of being ventilated at the time of diagnosis
 - Higher rates of mortality
- But all this data is associative



82

Transfusions and NEC

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 - Lower gestation
 - Lower birth weight
 - Higher risk of PDA
 - Higher risk of being ventilated at the time of diagnosis
 - Higher rates of mortality
- But all this data is associative



83

Transfusions and NEC: Possible mechanism

Original Investigation

JAMA

Association of Red Blood Cell Transfusion, Anemia, and Necrotizing Enterocolitis in Very Low-Birth-Weight Infants

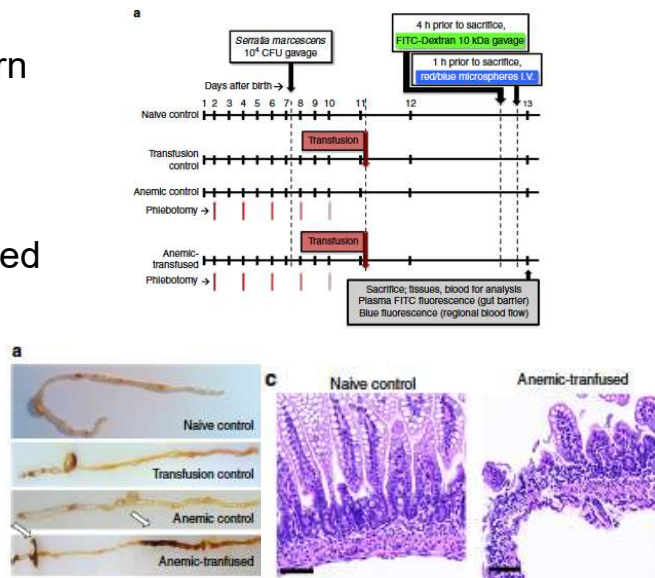
Ravi M. Patel, MD, MSc; Andrea Knezevic, MS; Neeta Shenvi, MS; Michael Hinkes, MD; Sarah Keene, MD; John D. Roback, MD, PhD; Kirk A. Easley, MAPStat; Cassandra D. Josephson, MD

| Risk factors | NEC | | | Mortality | | |
|--|------|------------|-------------------------------|-----------|------------|-------------------------------|
| | CSHR | 95% CI | P (Reliability ^a) | CSHR | 95% CI | P (Reliability ^a) |
| Birth weight (per 100g increase) | 0.68 | 0.56, 0.83 | <0.001 (97%) | 0.67 | 0.51, 0.88 | 0.004 (96%) |
| Received RBC transfusion in a given week ^b | 0.52 | 0.19, 1.40 | 0.19 (31%) | 1.14 | 0.26, 5.08 | 0.87 (12%) |
| Severe anemia (Hb ≤ 8 g/dL) in a given week ^a | 6.32 | 1.94, 20.6 | 0.002 (71%) | 1.74 | 0.43, 7.08 | 0.44 (25%) |
| Days of breast milk feeding in 1st 10 days (per 1 day increase) | 1.12 | 1.01, 1.24 | 0.04 (42%) | 0.87 | 0.78, 0.98 | 0.02 (41%) |
| SNAP score on day of birth (per 1 point increase) | 0.97 | 0.90, 1.05 | 0.45 (19%) | 1.12 | 1.02, 1.23 | 0.02 (59%) |
| Days of antibiotic treatment in 1st 10 days (per 1 day increase) | 1.04 | 0.93, 1.18 | 0.49 (5%) | 0.99 | 0.82, 1.19 | 0.90 (10%) |

84

Transfusions and NEC: Possible mechanism

- Serial phlebotomy of newborn mice
 - 40µl draws
- Transfusion with leukoreduced adult mouse blood
 - 20ml/kg
- 13-day experiment
- Dam fed throughout



MohanKumar *et al*, Nat Communications 2019

85

Transfusions and NEC: Possible mechanism



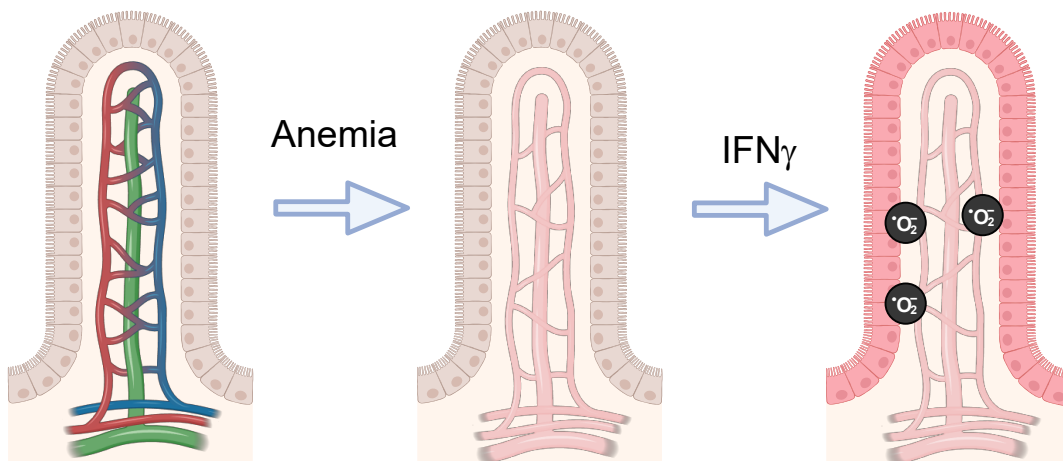
Anemia induces gut inflammation and injury in an animal model of preterm infants

Connie M. Arthur,¹ Demet Nalbant,³ Henry A. Feldman,⁵ Bejan J. Saeedi,¹ Jason Matthews,¹
Brian S. Robinson,¹ Nourine A. Kamili,¹ Ashley Bennett,¹ Gretchen A. Cress,³ Martha Sola-Visner,⁵
Rheinallt M. Jones,¹ M. Bridget Zimmerman,³ Andrew S. Neish,¹ Ravi M. Patel,² Peggy Nopoulos,⁴
Michael K. Georgieff,⁶ John D. Roback,¹ John A. Widness,³ Cassandra D. Josephson,^{1,2} and
Sean R. Stowell¹

- In preterm infants, the degree of anemia correlated with serum inflammation (Inf γ)
- In a mouse model, anemia induced intestinal hypoxia and upregulated macrophage HIF1 α
 - Increases Inf γ production

86

Transfusions and NEC: Possible mechanism



87

- What to eat
- Probiotics
- A lesson from blood
- **Antibiotics**



88

Why talk about antibiotics?

- Universally accepted associated risk factors include:
 - Dysregulated immune system/response
 - Abnormal (dysbiotic) microbiome
 - Immature intestine (including immunity and barrier)
- So, antibiotics are part of the supportive therapy

89

But which ones and how long?



90

Current practice is....muddy

- Current therapy covers anaerobic and gram-negative bacteria.
- IV ampicillin and gentamycin combined with metronidazole for 10-14 days are most common, however, there is great variability both in center, across centers, and internationally
- Cochrane reviews have not been able to determine optimal regimens

91

Some studies show an association with NEC



www.nature.com/pr

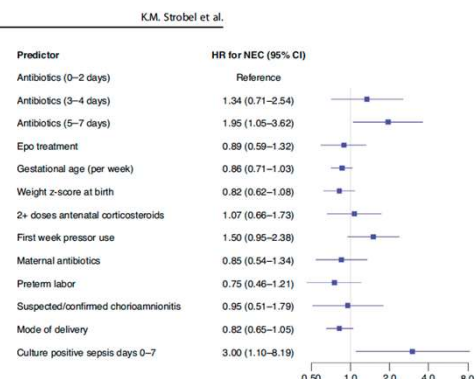
CLINICAL RESEARCH ARTICLE

Effect of early antibiotic exposure on necrotizing enterocolitis and growth in extremely preterm infants

Katie M. Strobel^{1,2}, Thomas R. Wood¹, Gregory C. Valentine¹, Olivia C. Brandon¹, D. Taylor Hendrixson^{1,2}, Dennis E. Mayock¹, Krystle M. Perez¹, Mihai Puia-Dumitrescu¹, Patrick J. Heagerty³ and Sandra E. Juul^{1,4}

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- Secondary analysis of the PENUT Trial
- 891 infants born 24-27 weeks of gestation
- After adjusting for maternal and infant factors
 - Peripartum antibiotics were not associated
 - Each additional day of antibiotics in the first week of life was associated with increased hazard for NEC



92

Mouse studies support the mechanism

- Newborn mice were treated with amp/gent for 10 days
- Induced a significant change in the microbiome
- Induced a loss of goblet and Paneth cells
- When challenged with bacteria, significantly more antibiotic exposed mice developed intestinal NEC-like injury



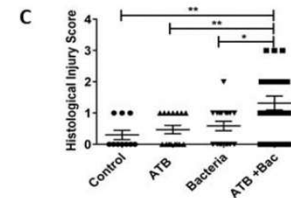
microorganisms



Article

Early Antibiotic Exposure Alters Intestinal Development and Increases Susceptibility to Necrotizing Enterocolitis: A Mechanistic Study

Hala Chaaban ^{1,*}, Maulin M. Patel ², Kathryn Burge ¹, Jeffrey V. Eckert ¹, Cristina Lupu ², Ravi S. Keshari ², Robert Silasi ², Girija Regmi ², Majoi Trammell ², David Dyer ², Steven J. McElroy ⁴ and Florea Lupu ²



D

| Incidence of NEC-like injury (scores ≥ 2) | Group, n (%) |
|---|---------------|
| Control | 0/10 (0) |
| ATB | 0/15 (0) |
| Bac | 1/17 (5.8%) |
| ATB + Bac | 12/22 (54.5%) |

93

While other studies show no association

CLINICAL RESEARCH ARTICLE

Check for updates

Early antibiotic exposure and necrotizing enterocolitis among preterm infants < 34 weeks' gestation

Yue Zhu^{1,2}, Shujuan Li^{2,3,12}, Siyuan Jiang^{2,3}, Weyin Yu², Yongfu Yu⁴, Zhenlang Lin⁵, Xiaoying Li⁶, Huiqing Sun⁷, Yan Mo⁸, Yihuang Huang⁹, Luyang Hong⁹, Joseph Y. Ting⁹, Shoo K. Lee¹⁰, Zhiping Li^{11,13}, Weibing Wang^{1,13}, Yun Cao^{2,3,13,15} and On behalf of the Reduction of Infection in Neonatal Intensive Care Units using the Evidence-based Practice for Improving Quality (REIN-EPIQ) Study Group

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- Multicenter cohort study of 24,926 infants
 - 20,164 had empiric antibiotics
- Found no association with NEC, death, or the combination
- However, the mean birthweight was around 1.6 kg so it is unclear what the smaller infant contribution was.

94

We need to be careful using QI to determine research questions

Journal of Pediatric Surgery 58 (2023) 1982–1989

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.sciencedirect.com/journal/journal-of-pediatric-surgery



Antimicrobial Stewardship in Neonates with Necrotizing Enterocolitis: A Quality Improvement Initiative

Devon Pace ^{a, e, 1}, Shale J. Mack ^e, Shannon Chan ^b, Samantha J. Mumford ^b, Lynn Fuchs ^c, Craig Shapiro ^d, Loren Berman ^{a, e, *}

^a Division of Pediatric Surgery, Nemours Children's Health, Wilmington, DE, USA
^b Department of Pharmacy, Nemours Children's Health, Wilmington, DE, USA
^c Division of Neonatology, Nemours Children's Health, Wilmington, DE, USA
^d Division of Infectious Disease, Nemours Children's Health, Wilmington, DE, USA
^e Sidney Kimmel Medical College of Thomas Jefferson University, Philadelphia, PA, USA

95

So Why Don't We Know More?



96

Animal models of NEC

- Original method of creating NEC developed by Barlow in the 1970's
 - Newborn rat pups were either dropper fed a simulated rat milk formula or dam fed
 - All animals were given an oral inoculation of *Klebsiella* at birth
 - Newborn rat pups were sealed in a plastic bag until cyanotic and limp (3-5 min) and then placing them in a cold stress at 7°C for 5 min daily.



97



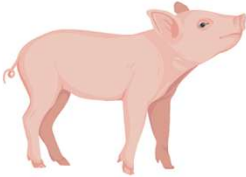
Animal models of NEC

- Deliver mice by cesarean section
- Gavage feed newborn mice prepared formula laced with bacteria every 3 hours
- Hypothermia/hypoxia stress twice a day
- 72 hours
- ~60% develop NEC



98

Animal models of NEC

| Mouse | Rat | Piglet |
|--|---|--|
|  |  |  |
| <ul style="list-style-type: none">• Fast breeders• Low cost• Transgenic models• Available antibodies/reagents• Correlation to human intestinal development | <ul style="list-style-type: none">• Fast breeders• Low cost• Easier to gavage than mouse | <ul style="list-style-type: none">• Preterm viability• Similar GI physiology/size/microbiome to human• Can mimic feeding practices and neonatal care• Can assess hemodynamics |
| <ul style="list-style-type: none">• Small size• Difficult to gavage | <ul style="list-style-type: none">• Sensitive to maternal milk• Lack of transgenic lines• Limited commercial antibodies, reagents• Tolerant to stressors | <ul style="list-style-type: none">• Expensive• Limited transgenic models• Limited molecular tools• Can develop global intestinal injury |


Also: hamsters, rabbits, dogs, quails, and non-human primates

Bautista et al, Front Pediat 2023

99


Current rodent models of NEC

Hypoxia Formula P4



bacteria or "trigger" + Q3 hour Formula + Q12 hour Hypoxia → NEC at 72 hours

Paneth Disruption P14



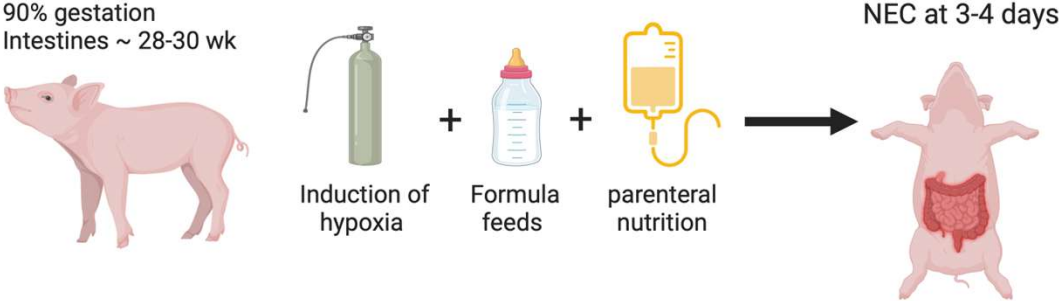
Dithizone + *Klebsiella* → NEC at 16 hours

Image Source: McElroy Lab

100

Current major piglet of NEC

90% gestation
Intestines ~ 28-30 wk



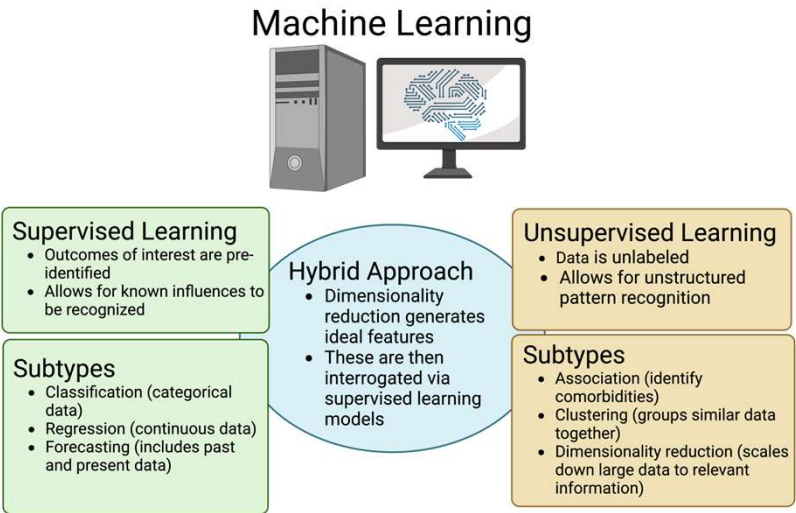
Induction of hypoxia + Formula feeds + parenteral nutrition → NEC at 3-4 days

Image Source: McElroy Lab

101

Machine learning models of NEC

Machine Learning



Supervised Learning

- Outcomes of interest are pre-identified
- Allows for known influences to be recognized

Subtypes

- Classification (categorical data)
- Regression (continuous data)
- Forecasting (includes past and present data)

Hybrid Approach

- Dimensionality reduction generates ideal features
- These are then interrogated via supervised learning models

Unsupervised Learning

- Data is unlabeled
- Allows for unstructured pattern recognition

Subtypes

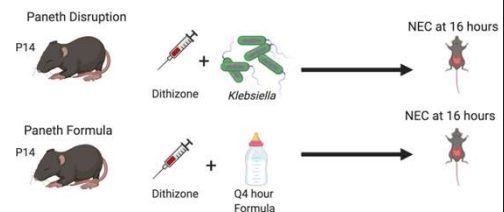
- Association (identify comorbidities)
- Clustering (groups similar data together)
- Dimensionality reduction (scales down large data to relevant information)

McElroy and Leuschow, *Front Pediat* 2023

102

McElroy lab model of NEC

- Use of 14-16 day old mice
 - Paneth cells don't develop until 7-10 days of life—epithelia is more similar to preterm infants at age of NEC
- Disrupt Paneth cells followed by induction of an inflammatory dysbiosis
- NEC-like Intestinal and systemic pathology 6-10 hours after dysbiosis



103

Summary

- NEC as an entity has been commonly described since the 1960's
- Current treatments have not substantially changed over the past 50+ years

104

Summary

- Only current known prevention is human milk
 - Mom's milk > Donor milk likely due to pasteurization and processing
 - Nutrition is of utmost importance.
 - To date, no data exists to support the claim that formula causes NEC
- Antibiotic therapy for NEC is generalized and supportive, but which are best and the appropriate duration are not known

105

Summary

- Probiotics appear to be beneficial, but which one, when, how much, etc is not known
- Transfusion associated NEC is only an association
 - Current data suggests that severe anemia followed by transfusions are a potential mechanism

106

