Probiotics and the intestinal microflora overtime and space

Eugenia Bezirtzoglou,MD,PhD

Professor in Microbiology

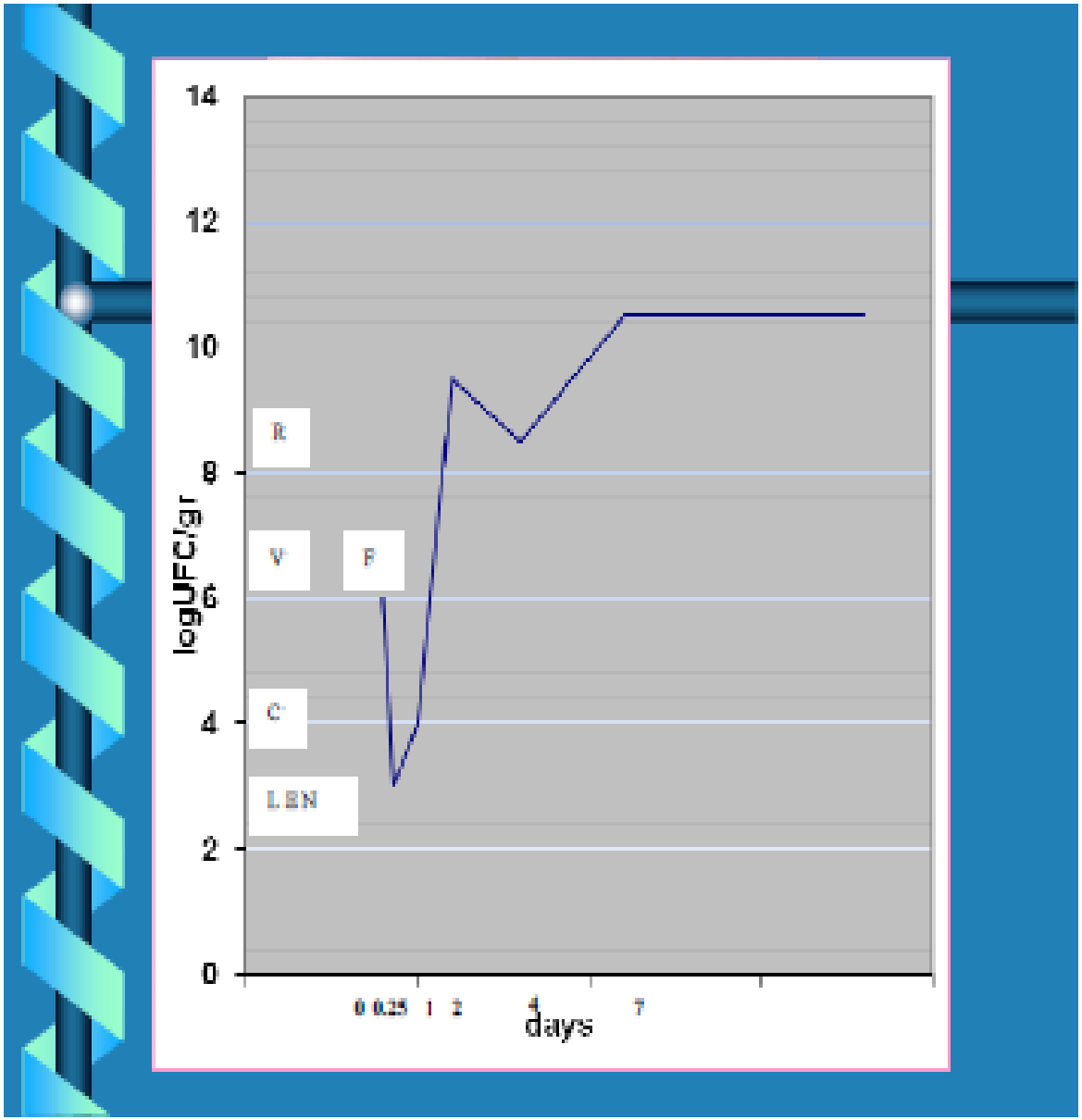
Laboratory of Microbiology,Biotechnology and Hygiene
School of Agricultural Development,
Section of Food Science and Technology,
Democritus University of Thrace, Greece

The human newborn devoid of bacteria before birth, is particularly prone to infection during the first days of life. This risk of infection is related to his insufficiently developed defense mechanisms and to exposure to a variety of microorganisms

NORMAL DEVELOPMENT OF THE INTESTINAL FLORA

A rapid and regular rise of the bacterial count was seen during the first week, the level of 10^9 CFU/g of feces being reached after one week.

In comparing the route of delivery in newborns coming from the same environment, the means of total bacterial counts were similar in both groups of infants during this period, but the breast-fed infants have shown higher total counts in the first days of life, due to contamination by the mother' s skin and milk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Indeed human milk frequently contains low amounts of non-pathogenic bacteria like *Streptococcus, Micrococcus, Lactobacillus, Staphylococcus, diphteroids* and *Bifidobacterium*.

The first bacteria colonizing the intestine in newborns delivered by **vaginal** delivery are of maternal origin mainly constituted from anaerobic bacteria.

In **caesarean** section infants, this flora is characterized by the lack of anaerobic bacteria, which do not survive in contact with air. Only micro-aerophilic microorganisms, facultative anaerobes and sporulate forms of bacteria like *Clostridium* colonizes generally the caesarean section newborn

Anaerobe

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The Intestinal Microflora During the First Weeks of Life

Eugenia Bezirtzoglou

F0	F6	F24	F48	F4	F7	F14
5.10^3 CFU/g	$5.\ 10^3$ CFU/g	$2.\ 10^9$ CFU/g	5.10^9 CFU/g	1.10^9 CFU/g	1.10^{10} CFU/g	5.10^9 CFU/g
Pacnes 5.10^3	Staphylococcus 5.10^3 Corynebacterium 5.10^3 E.coli 5.10^3 P. acnes 5.10^3	Staphylococcus 5.10^6 C. perfringens 5.10^6 Corynebacterium 5.10^5 E.coli 5.10^4 Streptococcus 5.10^4	Staphylococcus 5.10^9 Streptococcus 5.10^9 P. acnes 5.10^5 Ps. productus 5.10^3 C. perfringens 5.10^7 E. cloacae 5.10^7 E.coli 5.10^7 Corynebacterium 5.10^5 E. georgoviae 5.10^7	Streptococcus 5.10^7 Lactobacillus 5.10^7 Corynebacterium 5.10^7 E.coli 5.10^7 P. morbillorum 5.10^7 B.continuum 5.10^7 B.bifidum 5.10^6 E. ventriosum 5.10^6 Staphylococcus 5.10^6 B.breve 5.10^5 C.perfringens 5.10^3	Streptococcus 10^9 B. longum 10^9 B.adolescentis 10^9 B. breve 5.10^8 E. georgoviae 10^9 Corynebacterium 5.10^9 E.coli 5.10^9 B. infantis 5.10^7 Pc. prevotii 5.10^6 E. freundi 5.10^6 Staphylococcus 5.10^6 P. anaerobius 5.10^5	E. ventriosum 5.10^7 P. anaerobius 5.10^9 B. bifidum 5.10^7 E.georgoviae 5.10^7 Corynebacterium 5.10^7 Lactobacillus 5.10^7 Streptococcus 5.10^7 E.coli 5.10^7 Staphylococcus 5.10^9

In general, bacteria starts to appear in feces within few hours after birth.

E. coli, Staphylococcus and *Streptococcus* are isolated even in the very first stool in large numbers.

These first appearing microorganisms are probably creating, during the first few days of life, a reduced environment favorable to the immediate subsequent appearance of anaerobes.

Anaerobic bacteria belonging to the *Bacteroides, Clostridium* and *Bifidobacterium* genera can be detected in feces within two days, sometimes at high levels.

Among them, *Staphylococcus*, *Corynebacterium*, *Propionibacterium* and *Streptococcus* were the genera most commonly isolated from the newborn fecal flora at birth.

Under breast-feeding the flora is less diversified than under bottle-feeding. However, by about the end of the second year, the fecal flora of both groups of infants resembles that of adults.

IMPACT OF FEEDING ON THE INTESTINAL FLORA

In formula-fed infants, *Bacteroides* dominated among the anaerobes and high count of *Enterobacteriaceae* are found .

It has been more than an **axiom** that in breast-fed infants the intestinal flora is dominated by *Bifidobacterium*

The effect of human milk seemed to be the result of *B. bifidum* proliferation, in contrast to artificial alimentation that seemed to favour *C. perfringens* implantation .

Table 1: Colonization by *Clostridium perfringens* in relationship with infant feeding.

Day of life / Evolution	Infant	F0	F6	F24	F48	F4	F7	F14	Breast feeding ($n^+ = 5$)	Bottle and mixed feeding ($n^+ = 14$)
Decrease	BG	–	–	–	10^8	5.10^4	5.10^3	–	3	0
	SS	–	–	5.10^6	5.10^7	10^4	–	–		
	LC	–	–	–	5.10^8	5.10^7	5.10^6	5.10^4		
Apparition on the 14th day	SN	–	–	–	–	–	–	5.10^3	2(3.7* + 1.4)	4(7.2* + 1.9)
	PL	–	–	–	–	–	–	5.10^5		
	TL	–	–	–	–	–	–	5.10^6		
	AM	–	–	–	–	–	–	5.10^6		
	MN	–	–	–	–	–	–	5.10^4		
Maintenance	CC	–	–	–	5.10^6	5.10^6	5.10^6	5.10^6	0	8
	DN	–	–	–	5.10^4	–	5.10^5	5.10^5		
	DB	–	–	–	–	5.10^6	5.10^5	5.10^5		
	BF	–	–	–	–	–	5.10^3	5.10^7		
	CA	–	–	–	–	–	5.10^7	5.10^7		
	LS	–	–	–	–	–	5.10^6	5.10^5		
	CP	–	–	–	–	–	5.10^6	5.10^6		
	BC	–	5.10^4	–	–	–	5.10^7	5.10^5		

* Log_{10} mean counts of viable bacteria ± sem. ($p = 0.05$ Student test);
+ n = number of infants;
– No presence of *C. perfringens*;
Fecal flora of: F0 (0 h), F6 (6 h), F24 (24 h), F48 (48 h), F4 (four days), F7 (seven days) and F14 (14 days).

Table 2: Effect of feeding upon the frequency of *Bifidobacterium* isolation.

Day of life / Evolution		Infant	F0	F6	F24	F48	F4	F7	F14	Breast feeding (n = 5)	Bottle and mixed feeding (n = 14)	p*
Frequency on the 14th day*	*Bifidobacterium bifidum*	SS	-	-	-	-	5.10^6	-	5.10^7			
		LC	-	-	-	-	-	-	5.10^8	3		
		PL	-	-	-	-	-	-	5.10^5			0.0028
		BF	-	-	-	-	5.10^7	5.10^6	5.10^8		1	
	Other *Bifidobacterium* sp	LC	-	-	-	-	-	-	5.10^8			
		SN	-	-	-	-	5.10^6	-	5.10^7	2		
		CC	-	-	-	-	-	-	5.10^7			0.3
		BF	-	-	-	-	-	-	5.10^8		3	
		BP	-	-	-	-	-	-	5.10^5			

+ The probability was calculated according to Fisher's exact test;
* Before the 14th day, the anaerobic flora is often not implanted;
- No presence of *B. bifidum* or other *Bifidobacterium* sp.;
Fecal flora of: F0 (0 h), F6 (6 h), F24 (24 h), F48 (48 h), F4 (four days), F7 (seven days) and F14 (14 days).

Table 3: Correlation between *Clostridium perfringens* toward *Bifidobacterium bifidum* day by day.

Day of life / Infant	F0	F6	F24	F48	F4	F7	F14	Feeding
BG	–	–	–	$10^{8(1)}$ N.D(2)	5.10^4 N.D	5.10^3 N.D	–	M
SS	–	–	5.10^3 N.D	5.10^7 N.D	10^4 5.10^8	–	N.D 5.10^7	M
CC	–	–	–	5.10^6 N.D	5.10^3 N.D	5.10^3 N.D	5.10^6 N.D(5.10^{7*})	M+A
DN	–	–	–	5.10^7 N.D	–	5.10^3 N.D	5.10^6 N.D	A
DB	–	–	–	–	5.10^3 N.D	5.10^3 N.D	5.10^6 N.D	A
LC	–	–	–	5.10^5 N.D	5.10^7 N.D	5.10^3 N.D	5.10^4 5.10^7(5.10^{7*})	M
SN	–	–	–	–	N.D N.D (5.10^{6*})		5.10^3 N.D (5.10^{7*})	M
PL	–	–	–	–	–	–	5.10^7 5.10^8	M
LD	–	–	–	–	–	–	–	M+A
CM	–	–	–	–	–	–	–	M+A
TL	–	–	–	–	N.D 5.10^6	–	5.10^8 N.D	M+A
AM	–	–	–	–	–	–	5.10^8 N.D	M+A
BF	–	–	–	–	N.D 5.10^7	5.10^6 5.10^6	5.10^7 5.10^6 (5.10^{7*})	A
BP	–	–	–	–	–	–	N.D (5.10^{6*})	A
CA	–	–	–	–	–	5.10^7 N.D	5.10^7 N.D	A
LS	–	–	–	–	–	5.10^6 5.10^7	5.10^8 N.D	A
MN	–	–	–	–	–	–	5.10^6 N.D	A
CP	–	–	–	–	–	5.10^4 N.D	5.10^6 N.D	A
BC	–	5.10^6 N.D	–	–	–	5.10^7 N.D	5.10^7 N.D	A

(1) On the first line, the presence of *C. perfringens* is reported;
(2) On this second line, we note the presence of *B. bifidum*;
–: No *C. perfringens* and *B. bifidum* are detected;
N.D: Not determined, in presence of *B. bifidum* or *C. perfringens* correspondingly to which it is correlated.
*: Other *Bifidobacterium* sp.;
M: Maternal alimentation;
A: Artificial alimentation;
M+A: Mixed alimentation;
Fecal flora of: F0 (0 h), F6 (6 h), F24 (24 h), F48 (48 h), F4 (four days), F7 (seven days) and F14 (14 days).

An **antagonism** between these bacteria seems to be established in the newborn intestine, via the alimen tation

The composition and properties of human milk, such as high lactose, low casein and calcium phosphate and low buffering capacity, seem to favor the development of *Bifidobacterium*.

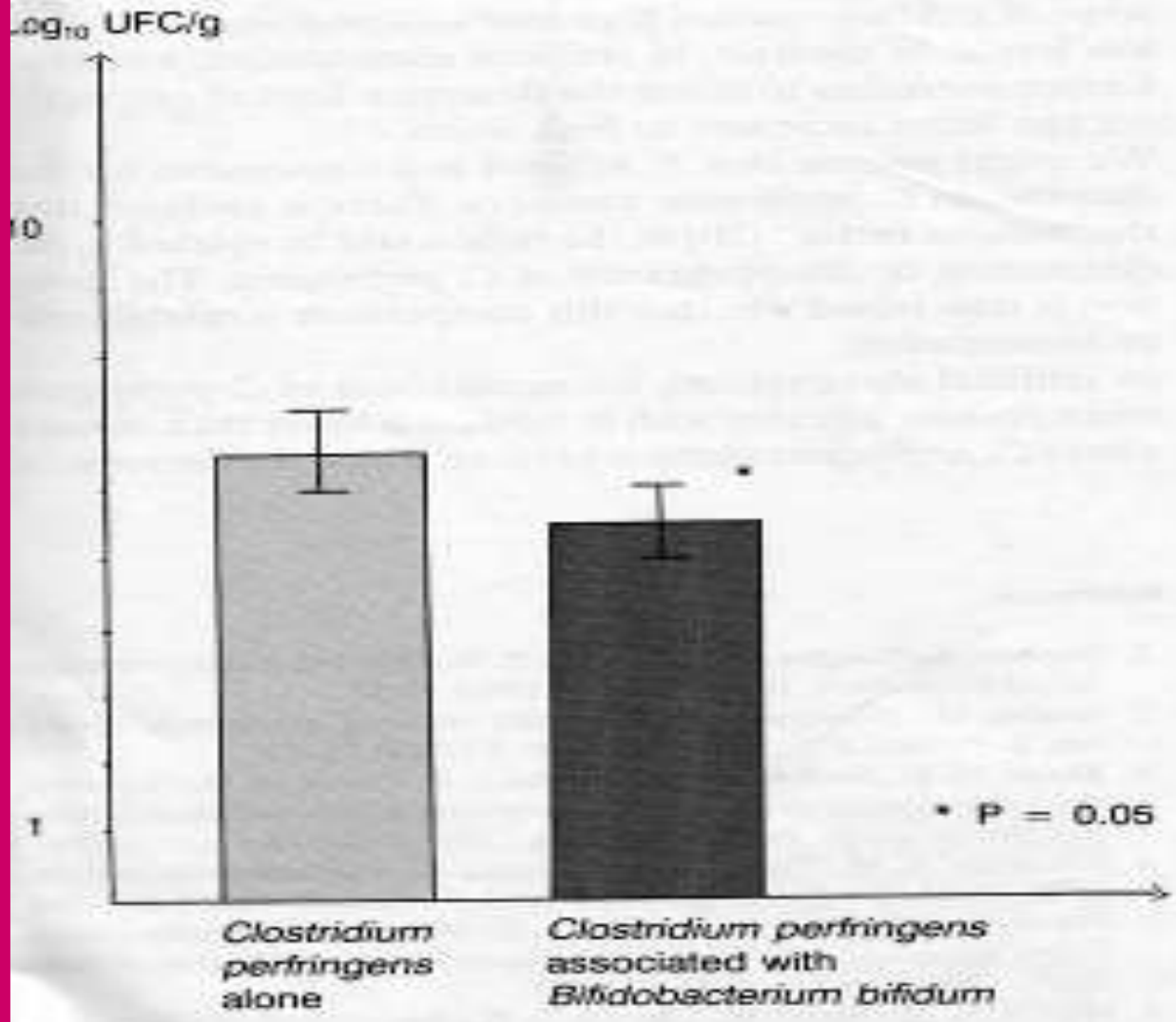


Figure 1: Presence of *Clostridium perfringens* in artificial alimentation.

In artificial alimentation, the mean count of *C. perfringens* when present together with *B. bifidum* was lower than in cases where *C. perfringens* alone was present.

Thus, the decrease of *C. perfringens* population in the newborn's intestine, seems to be associated with the presence of *B. bifidum* only, without any influence of alimentation.

This **antagonism** appears to exist directly between *B. bifidum* and *C. perfringens*, which can be intensified by the type of feeding. None of the other *Bifidobacteria* investigated led to the same decrease

IMPACT OF CAESAREAN SECTION ON THE INTESTINAL MICROFLORA

Newborns delivered by caesarean section begins life with a bacteriologically clean state, and offer an ideal model to better understand the installation of bacteria in the context of the neonate' s hospital.

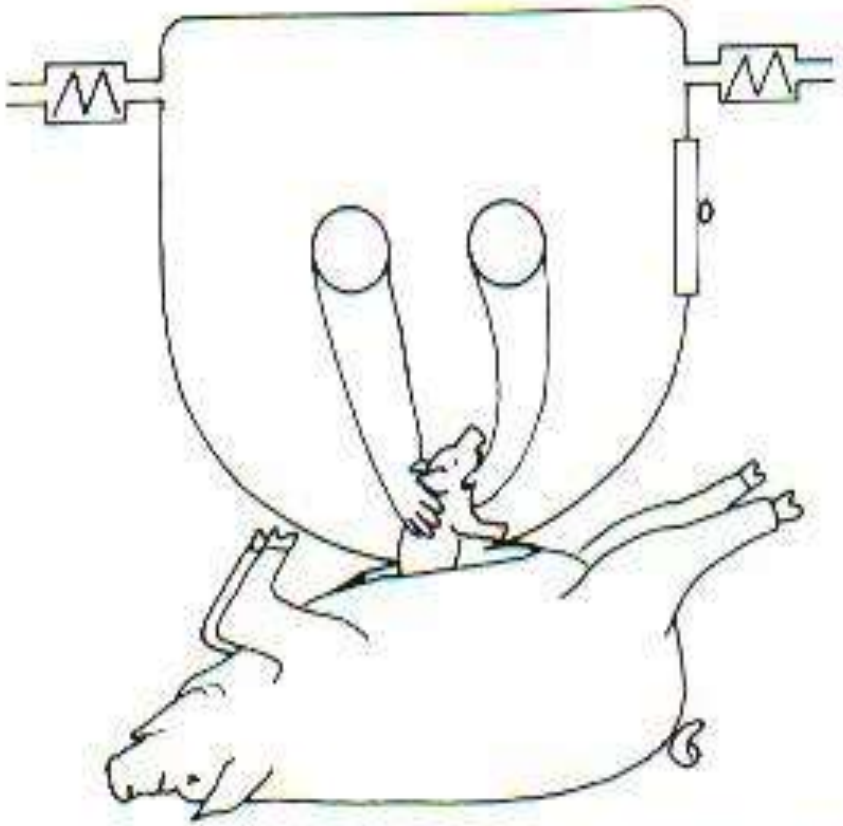
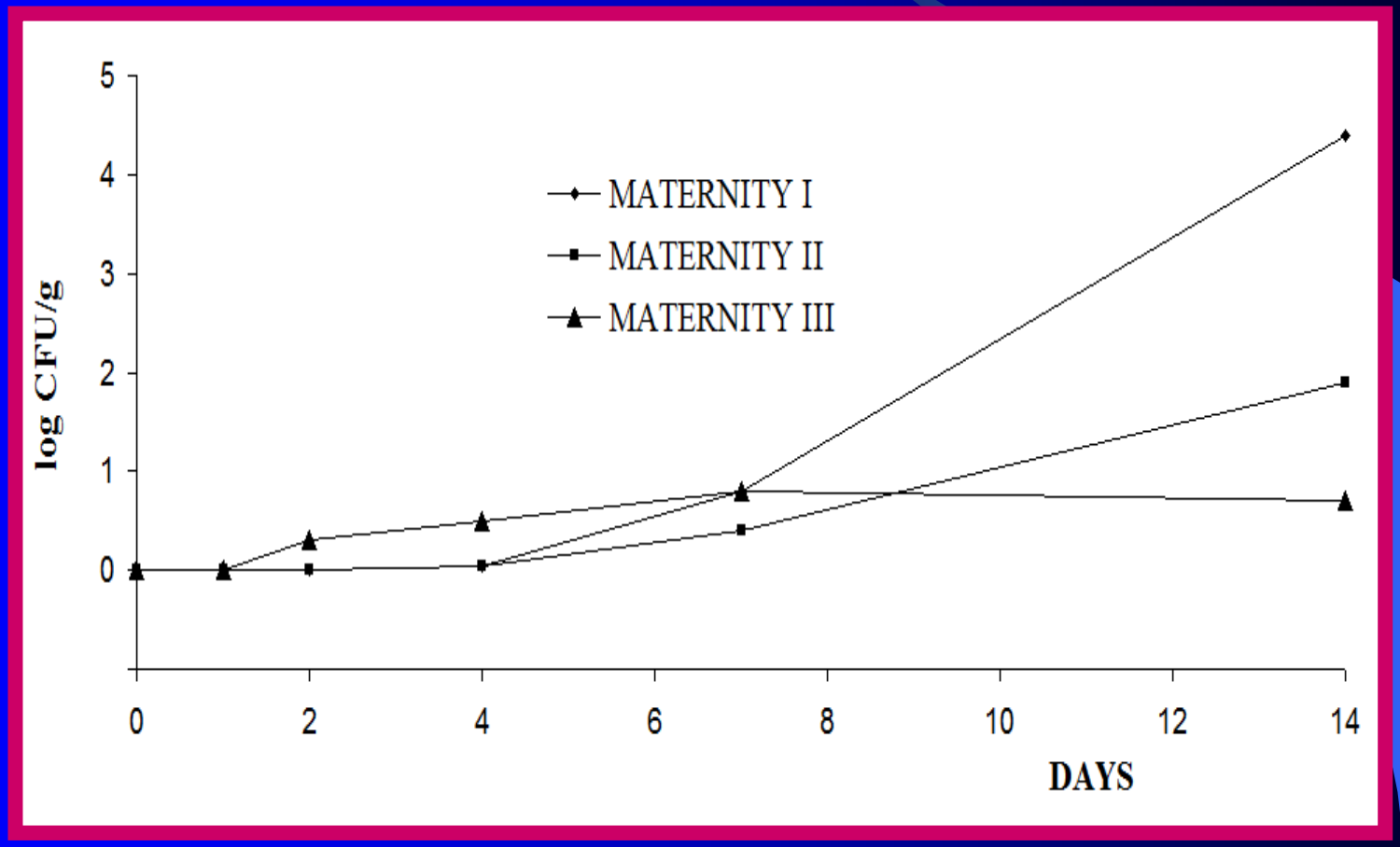


FIG. 5. — *Hystérotomie* ou césarienne aseptique. Le nouveau-né est passé directement de l'utérus de la mère dans un isolateur stérile dont le fond, collé sur le ventre de la mère, est incisé en même temps que les parois abdominale et utérine.

In babies born by caesarean section the first contact with bacteria is more fortuitous. These bacteria are coming from the **environment** or the the **hospital staff**. These bacteria coming from the hospital enviornment have a low colonization ability during the first 7 days of life . After the 7th day the bacterial counts in the newborn intestine are dependent on the environment.

Mean kinetic curve of CFU/g of feces in each maternity hospital



Anaerobic colonization is generally delayed but *Bifidobacterium* retrieval and *E. coli* presence was similar in vaginally and caesarean section delivered infants.

It is known actually that, *Bifidobacterium* species tend to cluster geographically in infants; this suggests that nosocomial sources may be more important than maternal ones .

An increase incidence of *C. perfringens*, is reported in relation with the hospital environment .

E.Bezirtzoglou and Romond B, Annals de Pediatrie

TABLEAU I. — **Evolution du *C. perfringens* dans les maternités I et II.**

TABLE I. — Changes in *C. perfringens* colonization over time in maternity wards I and II.

		Cas	Date d'apparition	Taux d'apparition	Evolution jusqu'au 14e jour	Allaitement*
MATERNITÉ I	PÉRIODE DES TRAVAUX	BG	48 h	5.10^3	Disparition	M
		SS	24 h	5.10^5	Disparition	M
		CC	48 h	5.10^8	Persistance au même taux	M + A
		DN	48 h	5.10^4	Persistance et augmentation	A
	2 ANS APRÈS	DB	4 j	5.10^6	Persistance	A
MATERNITÉ II		LC	48	5.10^8	Diminution du taux	M

* Allaitement : M : maternel ; A : artificiel ; M + A : mixte.
Types of feeding : M : breast-fed ; A : bottle-fed ; M + A : both breast-and bottle-fed.

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TABLEAU II. — Evolution du *C. perfringens* dans la maternité III.

TABLE II. — Changes in *C. perfringens* colonization over time in maternity ward III.

Cas	Date d'apparition	Taux d'apparition	Evolution jusqu'au 14e jour	Allaitement*
SN	14e j	5.10^2		M
PL	14e j	5.10^3		M
LD				M + A
CM				M + A
TL	14e j	5.10^3		M + A
AM	14e j	5.10^6		M + A
BF	7e j	5.10^3	Persistance et augmentation	A
BP	14e j	5.10^6		A
CA	7e j	5.10^7	Persistance au même taux	A
LS	7e j	5.10^8	Persistance mais diminution du taux	A
MN	14e j	5.10^4		A
CP	7e j	5.10^4	Persistance et augmentation	A
BC	6 h (?)	5.10^4 (?)	Disparition (6 h) Réapperition (7 j)	A

* Allaitement : M : maternel ; A : artificiel ; M + A : mixte.
Types of feeding : M : breast-fed ; A : bottle-fed ; M + A : both breast-and bottle-fed.

- During the last years new methodologies penetrate the scientific community to offer us assess to this approach and more accurate information.

Microbiota Profile in Feces of Breast- and Formula-Fed Newborns by Using Fluorescence *in situ* Hybridization (FISH)

Eugenia Bezirtzoglou, Arsenis Tsiotsias ,Gjalt W. Welling

Bacterial group	Stain or probe	Breast-fed newborns (n=6)			Formula-fed newborns (n=6)		
		Average ± SD cells/g of feces	average %		Average ± SD cells/g of feces *(Range)*	average %	
			DAPI	Bact338		DAPI	Bact338
Total cells	DAPI	$2.4 \times 10^{10} \pm 1.33 \times 10^{10}$	100		$2.1 \times 10^{10} \pm 1.3 \times 10^{10}$	100	
Total bacteria	Bact338	$2.7 \times 10^{10} \pm 1.82 \times 10^{10}$	102.9	100	$2.1 \times 10^{10} \pm 1.66 \times 10^{10}$	96.1	100
Bifidobacterium	Bif164	$1.8 \times 10^{10} \pm 1.07 \times 10^{10}$	72.3	69.1	$6.9 \times 10^{9} \pm 6.42 \times 10^{9}$	31.2	32.1
Bacteroides/Prevotella group	Bac303	$2.8 \times 10^{9} \pm 2.09 \times 10^{9}$	11.9	12.1	$6.5 \times 10^{9} \pm 5.62 \times 10^{9}$	28.7	28.9
Atopobium group	Ato291	$3.4 \times 10^{8} \pm 3.69 \times 10^{8}$	1.2	1.1	$1.3 \times 10^{9} \pm 1.36 \times 10^{9}$	6.8	7.5
E. coli and related species	EC1531	$8.9 \times 10^{8} \pm 8.24 \times 10^{8}$	2.9	3.0	$3.7 \times 10^{8} \pm 3.06 \times 10^{8}$	2.4	3.0
Veillonella	Veil223	$< 8.9 \times 10^{5}$	< 0.01	< 0.01	$1.6 \times 10^{8(a)} \pm 2.82 \times 10^{8}$	0.9	0.8
Clostridium group	Chis150/Clit135	$< 8.9 \times 10^{5}$	< 0.01	< 0.01	$6.7 \times 10^{7(a)} \pm 5.54 \times 10^{7}$	0.3	0.3
Eubacterium/Clostridium group	Erec482	$< 8.9 \times 10^{5}$	< 0.01	< 0.01	$1.8 \times 10^{8(a)} \pm 1.08 \times 10^{8}$	0.9	0.9
Lactobacillus/Enterococcus group	Lab158	$6.7 \times 10^{7} \pm 5.91 \times 10^{7}$	0.6	0.6	$2.1 \times 10^{7} \pm 8.3 \times 10^{6}$	0.1	0.1
E. faecium/E. faecalis	Enfl3/Enfm2	$< 8.9 \times 10^{5}$	< 0.01	< 0.01	$1.6 \times 10^{7} \pm 1.75 \times 10^{7}$	0.1	0.1
Streptococcus/Lactococcus group	Strc493	$5.2 \times 10^{6} \pm 8.22 \times 10^{6}$	0.07	0.08	$7.5 \times 10^{7(b)} \pm 8 \times 10^{7}$	0.4	0.5
Staphylococcus group	Stau72	$1.0 \times 10^{7} \pm 1.11 \times 10^{7}$	0.1	0.1	$5.9 \times 10^{7(a)} \pm 2.61 \times 10^{7}$	0.4	0.4

IMPACT OF HOSPITALIZATION ON THE INTESTINAL FLORA

Hospitalization is incriminated to change the normal microflora. Changes in intestinal microflora as regards antimicrobial resistance of the bacteria and changes of bacteria species is noted .

Also, in newborns, intestinal colonization by *Klebsiella*, as well as by other *Enterobacteriaceae* occurs.It is much more pronounced after caesarean section.

It has been observed a delay in *Bifidobacterium* colonization, a predominance of *Bacteroides*, especially after vaginal delivery and an increased incidence of *Clostridium* species.

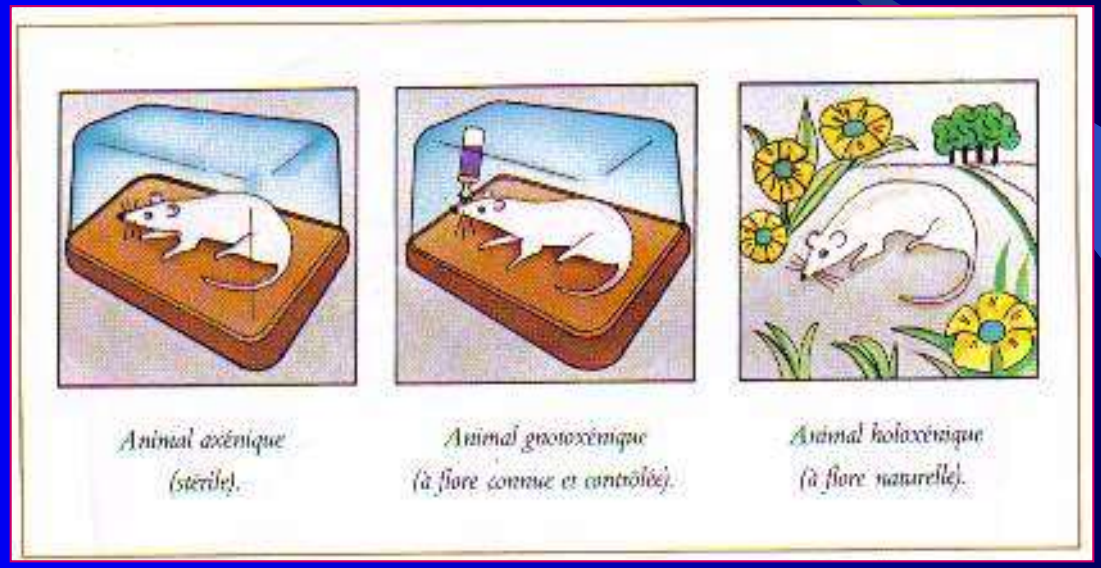
No differences seems to exist between term and preterm infants.

FACTORS INTERVENING ON THE INTESTINAL FLORA

Independing of its origin, the intestinal flora seems to "be transient" confraiting numerous difficulties in its installation.
Important microbial and biochemical events are taking place.

Newborn **intestine** may be an important reason.

Germfree animals with no microflora are extremely susceptible to colonization with pathogenic microorganisms whereas conventional animals with an intact flora are quite resistant .



Animal axénique (stérile).

Animal gnotoxénique (à flore connue et contrôlée).

Animal holoxénique (à flore naturelle).

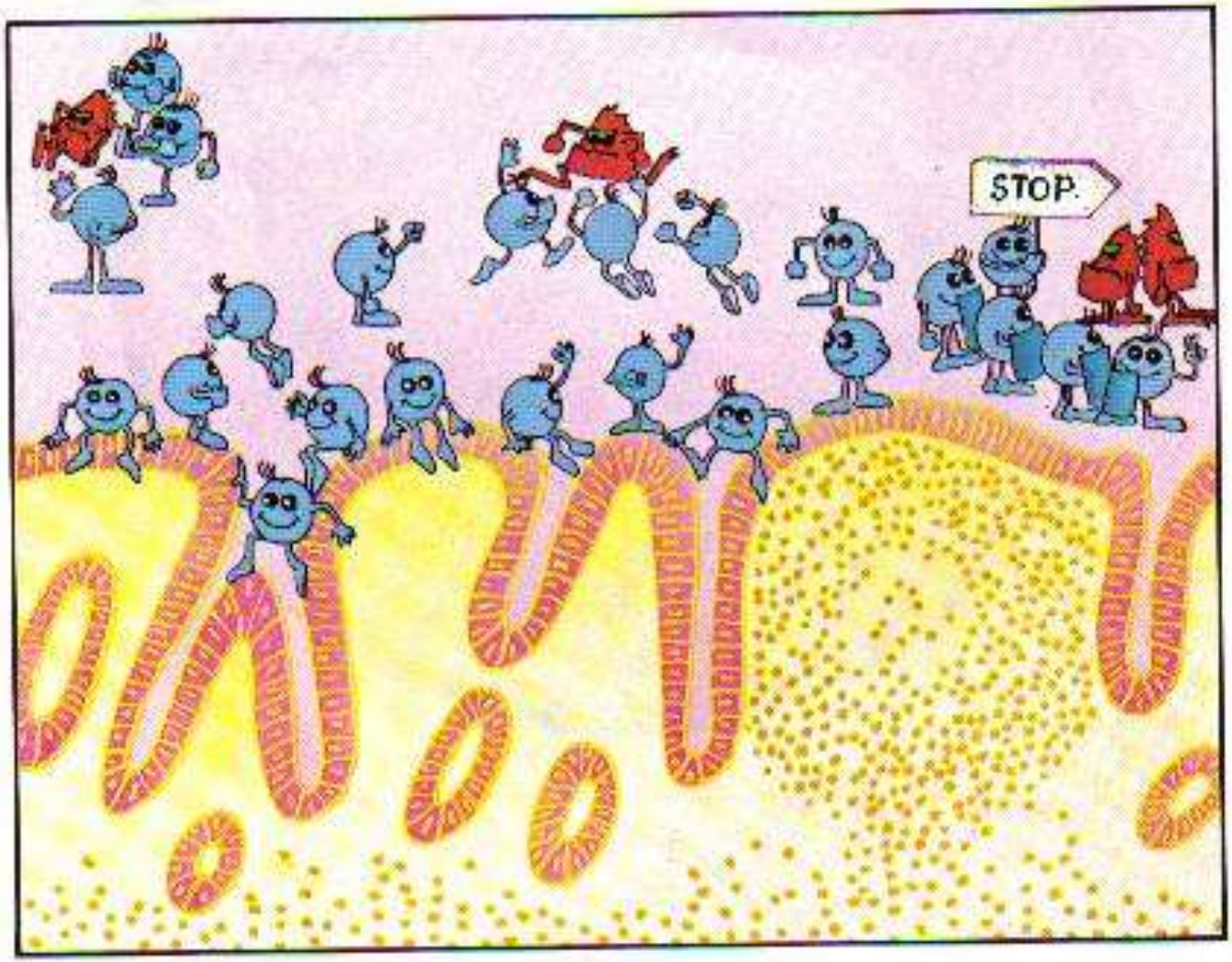
Local host defence factors in the human newborn prevent against colonization by pathogenic microorganisms at the mucous membranes, when first exposed to the microbial world.

Secretory IgA antibodies are not detected in newborns stools until the fourth week of life.

Mucosal phagocytes, lactoferrin, lysozyme, mucus glucoprotein, fibronectin may be involved.

- The variation among different species or even among different strains within a species reflect the complexity of the genetic polymorphism which regulate the immune system functions.
- Additionally factors such as, gender, particular habits, smoking, alcohol consumption, diet, religion ,age ,gender, precedent infections and vaccinations must be involved
- .Hormonal profile and stress seems to be associated to the integrity microflora and inducing immune system alterations

It is known that an intact indigenous flora represents a formidable barrier to the establishment of pathogenic populations on host surfaces; this phenomenon is called **"bacterial antagonism", "bacterial interference"** or more commonly "**colonization resistance**".



La barrière microbienne

This **"barrier effect**" includes many activities as, production of bacteriocins by the indigenous flora components, production of metabolic and products which will be toxic for pathogens, conditions inhibitory to pathogens, such as low pH, depletion of nutrients required for multiplication of pathogens.

For description of the interactions found between the host and its microflora, two new terms, namely **GAC** and **MAC**, were newly brought in to use by Profeesor Midtvedt at Karolinska Institutet

A list of MACs (= microflora associated characteristics), contains several parameters; **degradation of tryptic activity, conversion of cholesterol to coprostanol, conversion of bilirubin to urobilinogen, absence of β-aspartylglycine, breakdown of mucin.**

When microbes are totaly absent, as in Germ-free animals, any recording made of the functions and structures studied can be classified as a GAC (= germfree animal characteristics).

Antimicrobial drugs can change MAC to GAC

- Many tissues within the body and bacteria are known to posses some CYP activity,
- the prevailing dogma is that the intestine is associated to an important extend with CYP metabolism, as it is responsible for the extrahepatic metabolism

- Based on the fact that many intestinal bacterial strains possess P450 enzymes,
- the question is raised then if live probiotics express a P450 activity, which could eventually influence the drug metabolism and bioavailabity.

- In conclusion, the benefic microflora dominated by Bifidobacteria and Lactobacilli supports the concept of a healthy intestinal system and promote its abilities to modify beneficially the gut microbiota
- Immune system modulation
- Regulation of gut motility and Maintenance of mucosal integrity
- Decreased incidence and duration of diarrhea
- Reducing the risk of colon cancer
- Developing antimutagenic and anti - allergic activities
- Preventing of osteoporosis
- Hypocholestaemic action
- Feeling of well - being

The aim of this review was to bring together a good deal of the so numerous data on infant intestinal flora. Controlling mechanisms and host factors that influence bacterial succession and the effect of alimentation contribute more to the complexity of the problem.

Another problem is that several bacterial species present a different geographic distribution, as it is the case of *Bifidobacterium sp.*

It is obvious, that cooperative research in different parts of the world needed to widen our knowledge about the global ecology of different species, colonizing the newborn intestine and particularly of the source and mode of transmission.

ΕΥΧΑΡΙΣΤΩ ΠΟΛΥ ΓΙΑ ΤΗΝ ΠΡΟΣΟΧΗ ΣΑΣ

That’s all Greek to you!!!

THANK YOU VERY MUCH FOR YOUR ATTENTION