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# IMPLANTATION AND DEVELOPMENT OF THE GUT FLORA IN THE NEWBORN ANIMAL

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**Abstract**

The newborn mammal, germfree in the mother's uterus, steps in complex microbial environment as soon as born. Bacterial development in the digestive cavities of the newborn animal, from the environmental bacteria, occurs very quickly. Within three hours after birth a small microbial population is present in the piglet, baby mouse or human baby. Within twelve hours after birth, dominant microbial population of the newborn animal can be as important as that of the adult animal. In any case, this highest level is reached within 24 h after birth. Studying several animal species, one observes a certain diversity in the steps of establishment of the principal bacterial groups. For instance, facultative anaerobic bacteria appear before strictly anaerobic bacteria in the young mouse, whereas the opposite situation happens in the young hare. In the calf and the piglet, strictly and facultative bacteria establish approximatively at the same time. On day one after birth *E. coli* and *Streptococci* establish in the human infant, and also species belonging to genus *Bacteroides* and *Bifidobacterium*. This last genus becomes dominant from 4 to 5 days after birth. The newborn successively meets three different bacterial ecosystems: maternal vagina, maternal feces and finally environment where it is reared. Each of these three ecosystems probably takes a role in the elaboration of the newborn animals flora, but in proportion difficult to estimate. Any way, one can state that the newborn possess powerful systems to sort out certain specific bacterial species among those constituting the environmental population to which he is exposed. Indeed, the first bacterial species to establish in the newborn animal are not those dominating the three different ecosystems met, and therefore those he mostly can encounter. In the digestive tract of the newborn human baby we have been able to prove the existence of exogenous bacteria in transit, that will be eliminate without development. Mechanisms involved in the regulation of the sequence of establishment of the digestive tract microflora still are not well known. The first factor one can suggest is diet composition, that is maternal milk as far as newborn animals are concerned. In different experimental systems, it is directly proved that milk composition directly influences the establishment possibilities of one or another bacteria. However this didn't often lead to the precise identification of the milk factors involved. As well, it is known that maternal diet can influence the establishment of certain bacterial species in the newborn animal. The immunological state of the mother, on which depends the composition of colostrum given to the young could interfere in the establishment sequence of its flora. In fact if colostrum part in preventing infection from intestinal source is unquestionable, there is no proof of its action on the microflora in the lumen of the digestive tract. Interactions between different species of bacteria can also interfere in this establishment sequence. These interactions exist between different species of bacteria or between bacterial strains belonging to same species. For instance, we have demonstrated that early inoculation of certain *E. coli* strains had an antagonistic effect upon the proliferation of other antibioresistant plasmids carrier *E. coli* strains. Microbial interactions can be employed in practice by inoculating newborn animals with microbial flora able to eliminate pathogenous bacteria. Such a phenomenon has been described in the young hare.

The young mammal, which is axenic in the mother uterus is suddenly plunged at birth into a complex bacterial environment. During its passage through the maternal vagina it will meet the first bacteria, then at the expulsion, it often comes into contact with the enormous bacterial population of the mothers' feces. Finally, after birth it will probably swallow or inhale all the bacterial species present in its rearing environment.

It may be considered therefore that the newborn is very likely to meet potentially pathogenic bacterial species and, accordingly, the survival of the animal species depends on the very early establishment of defence systems towards the most detrimental part of the bacterial environment.

The purpose of the present paper is to make an evaluation of our present knowledge o the establishment of the flora in the human and animal newborn in «normal» conditions, i.e., when the dam does not exhibit any pathological disorder at parturition.

*Duration of the microflora establishment in the young digestive tract*

The establishment of a bacterial flora in the young digestive cavities is very fast. When the fecal dominant flora is studied in a series of piglets of different ages after birth, it is observed that few bacteria are enumerated at the third hour of life. After 10 to 12 h, the numbers of these bacteria have grown and the size of the dominant population reaches a high level with $10^8$ to $10^9$ bacteria per gram of feces. In the young mouse born of gnotobiotic dams harboring a flora of facultatively anaerobic bacteria it is observed that all the animals of 24 h of age already possess a dominant flora established at a level which will not change during several weeks of observation (Ducluzeau and Raibaud, 1974).

In a study made on the human infant we studied the flora of 14 samples of meconium discharged within 18 to 51 h. A single sample was sterile and 10 already possessed a dominant flora whose level exceeded $10^9$ bacteria per gram (Patte *et al.*, 1980). In another study, 11 out of 15 samples of meconium studied within 24 h after birth were found to be sterile. In the others the dominant flora exceeded $10^8$ bacteria per gram. After 48 h all the infants had a fecal flora whose level was higher than $10^8$ bacteria per gram (Lejeune *et al.*, 1981).

It may be concluded that the gut flora of young mammals becomes established between 24 and 48 h of age at a level which is already maximum and will remain almost stable during their whole life.

*Nature of the first established strains*

Qualitatively the flora which becomes established in the newborn is very different from that of the adult. Moreover, there are differences in the order of implantation of the main bacterial groups according to the animal species. In the young mouse, facultatively anaerobic bacteria such as *Lactobacillus, Streptococcus, Escherichia* become established very early (Schaedler *et al.*, 1965; Lee and Gemmell, 1972; Ducluzeau and Raibaud, 1973; Chopin *et al.*, 1974), while the appearance of strictly anaerobic bacteria which will eventually be dominant occurs later from the third week of life (Chopin *et al.*, 1974; Ducluzeau *et al.*, 1974). The opposite is observed in the young hare and the young rabbit. Strictly anaerobic bacteria appear from the first hours of life and dominate the *Streptococcus,* the only facultatively anaerobic bacteria to be present at that age (Gouet and Fonty, 1973; Ducluzeau *et al.*, 1975). The piglet represents an intermediate case with the simultaneous establishment from the first day both facultatively anaerobic bacteria, mainly *Streptococcus,* and *Escherichia,* and strictly anaerobic bacteria belonging to *Clostridium, Fusobacterium, Peptostreptococcus* and sometimes bacteroides genera (Wilbur *et al.*, 1960; Smith and Crabb, 1961; Pesti, 1962).

In the human infant, fed the mother's milk, the same situation is observed: almost simultaneous establishment of facultatively anaerobic genera *Escherichia, Streptococcus* and strictly anaerobic genera *Bacteroides* and *Bifidobacterium*, the latter genus becoming dominant at the end of the first week (Seeliger and Werner, 1963; Raibaud *et al.*, 1975; Hudault *et al.*, 1976; Ducluzeau *et al.*, 1982).

*Origin of the newborn microbial flora*

During the process of birth the newborn meets successively several different microbial ecosystems: the mother vagina, the mother feces with which it is nearly always in contact at a given moment, and finally the environment: flora of the room where it is kept and flora of the dam udder. It may be though *a priori* that each of these ecosystems is liable to contribute to the creation of the newborn gut flora. In fact, it is observed that the newborn is provided with powerful selective systems allowing it to choose only some bacterial species among all the bacteria of these ecosystems. The first bacteria which become established in the digestive tract actually come from the dam or from the environment, but they are not the most abundant of the ecosystems met by the young. The following experiment illustrates this capacity of selection of the young. All the meconiums and feces from a human infant were successively collected from birth to 35 h of age. A qualitative and quantitative analysis of the microbial flora was performed on a fraction of each meconium or feces and another fraction was inoculated immediately after sampling into an axenic mouse. Seven days after the cecal flora of each mouse was analysed. In the mouse inoculated with the meconium discharged at the 5th hour, the presence of a complex flora was observed. It contained in particular strictly anaerobic belonging to genera *Eubacterium* and *Clostridium* that were not recovered in the corresponding meconium and which did not appear in the infant flora 30 h later. It may be concluded that these bacteria were present in the infant gut at the 5th h but in a too small number to be directly detected, or that they were just in transit without never becoming established (Patte *et al.*, 1980).

*Mechanisms controlling the establishment of the flora in the young*

One of the main factors liable to account for the selection exerted by the newborn among all the bacteria met, is its dietary regimen, i.e., in that particular case: milk. The digestive content of the young mammal is favourable to the growth of some bacteria and unfavourable to others. For instance, it was observed in young mice born to gnotobiotic dams harboring only *E. coli* and *S. flexneri* that these bacteria were implanted in high numbers in all the animals after 12 h (Ducluzeau and Raibaud, 1973). Conversely, when dams only harbored strictly anaerobic, such as *Bacteroides* and *Clostridium,* these bacteria were detected in the young only between 10 and 15 days of age, i.e. when the young began to intake solid food in addition to milk (Ducluzeau *et al*, 1974).

Differences were observed a long time ago between floras from human infants fed either the mother's milk or humanized cow's milk. In the presence of maternal milk, an early establishment of *Escherichia coli* and *Streptococcus* is observed and after five or six days an almost exclusive domination of genus *Bifidobacterium*. In the infant fed milk replacer the flora is at once more varied: the *E. coli* species is accompanied by many others enterobacteria often belonging to genera *Enterobacter (or), Proteus* and in the dominant flora many other strictly anaerobic bacterial genera are found later such as *Clostridium, Eubacterium, Peptostreptococcus* or *Bacteroides* at a level equal or superior to that of the *Bifidobacterium* population (Ducluzeau *et al.*, 1982). However, the milk factors responsible for the observed differences have not yet been accurately identified. Moreover, the mechanism of action of such factors seems to be very complex as shown by the demonstration of the «remanence» effect: if an axenic mouse only fed with doe rabbit's milk is inoculated with a strain of *Clostridium* this strain becomes established, if the mouse is fed a commercial diet this strain does not become established. But if the strain of *Clostridium* is inoculated into a mouse fed with milk and if the latter is replaced by a non-permissive commercial diet it is observed that the strain remains at a high level in the mouse digestive tract for several months (Ducluzeau *et al.*, 1981). This shows that the diet, even if it is given for a short period, is liable to cause long term changes in the microbial pattern.

The dam's diet may also lead to variations in the implantation of some bacteria in the suckled young, as illustrated by the following experiment. One group of gnotobiotic lactating mice were fed a semi-synthetic diet (dams S), others a commercial diet (dams C). It was observed that a strain of *Lactobacillus sp.* became established at similar levels in their digestive tract. On the other hand, this strain of *Lactobacillus* was not detected during the lactation period in the young from dams S, while it became established from the first days in the young from dams C (Lhuillery *et al.*, 1981). Accordingly, the maternal milk might include factors favoring or inhibiting the bacterial growth. Their presence in the maternal milk might depend on the dam's diet.

The immune status of the dam which affects the composition of the colostrum given to the young might also influence the order of establishment of the bacteria in the young. Colostrum, undoubtedly, plays a role in the fight against microbial infection of digestive origin, but it was never demonstrated that it could have any effect on the microflora of the gut lumen. Immunization of the dams against different bacterial species did not prevent the further establishment of these bacteria in their young (Ducluzeau and Raibaud, 1973). We studied the establishment of the fecal flora between birth and 48 h of age in a litter of conventional piglets divided into two groups: in the first one the animals were left with their dam and suckled colostrum and milk, in the other, animals were taken off at birth, housed in a cage inside the dam's pen and received sterile cow's milk. We did not observe any difference in the systematic order of implantation of the first bacteria between both groups of piglets.

However, bacterial interactions certainly play an important role in the order of establishment of the flora. The early development of some bacteria in the digestive tract prevents proliferation of other bacteria. This was demonstrated experimentally in the human gnotobiotic newborn. It was observed that a strain of *E. coli* of human origin was capable of eliminating from the digestive tract a strain of *Lactobacillus casei* derived from a commercial preparation and previously established in the axenic newborn (Hudault *et al.*, 1976). In human newborn it was shown that the inoculation of a strain of *E.*

*coli* without plasmid within two hours following birth led to the elimination or to a marked decrease of the *E. coli* population carrying plasmids of resistance to antibiotics, while these strains were frequently dominant in the control (Duval-Iflah *et al.*, 1982).

The order of establishment of the bacteria in the young is probably extremely important for its survival as shown by an experiment made in the young hare. In this animal, the administration of a mixture of antibiotics within two days after birth enabled the survival up to weaning by eliminating diarrhoea due to toxinogenic *Clostridium* which are frequent causes of mortality in this species during the first ten days. On the other hand, the disturbance by this small administration of antibiotics of the initial establishment of the flora led almost inevitably to the death of animals treated at weaning, thus long after the end of the antibiotic treatment (Ducluzeau *et al.*, 1975). The practical application of the ideas of bacterial interaction was found to be more useful in the above case than the use of antibiotics. If the young hare was inoculated within the hour following birth with a complex flora derived from a healthy young hare, it was observed that it was totally protected from neonatal diarrhoea without further pathological manifestation at weaning. This protective flora, at present preserved in gnotobiotic mice, failed to be simplified up to now to obtain a mixture of pure strains liable to be cultured *in vitro* (Ducluzeau *et al.*, 1981). A protective effect was also demonstrated in the axenic newborn piglet: the early inoculation of a selected strain of *E. coli* of pig origin was found to protect the animals from the further development of toxinogenic strains of *E. coli* provided that the latter were not capable of adhering to the gut mucosa.

## Conclusions

The establishment of the microflora in the newborn mammal is a complex process which does not always take place in the best conditions as shown by the frequency of the digestive disorders in the young in many animal species. The control of this establishment would lead to a noticeable progress in the rearing techniques of laboratory and farm animals, as well as of human infants. Despite the diversity of the means available to achieve this control, we still lack data to choose the most efficient ones. However, it may be excepted that in the near future each human or animal newborn will be given the most appropriate bacterial mixture enabling it to resist the unfavourable components of its environment, as well as the milk necessary to the early establishment of the bacteria of this mixture.

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**Question**

*From Dr. Vannier to Dr. Ducluzeau*

What factors in the milk other than immune ones could exert a selection on the microflora of the digestive tract of the new-born ?

What is the role of the bacteriophages for the regulation of the bacterial flora in the digestive tract ?

**Answer**

The milk factors responsible for microflora selection in the young have long been unknown. For instance, the various artificial milks given to human babies do not allow the establishment of a flora similar to the flora of suckling babies. We have demonstrated in experimental animals that nature of milk liquid fatty acids probably plays a role in the implantation Any role of the bacteriophages in the digestive tract has been experimentally demonstrated.

**Question**

*From Dr. Larvor to Dr. Ducluzeau*

Question 1

What were the barrier flora inoculation conditions in young hares ?

Question 2

I have never heard of any experiment that clearly showed the preventive effect of barrier flora in pathology ; Is this true ?

**Answer**

1. Inoculation was performed very early : two hours after birth.

2. There is few conclusive data on this subject. In pigs the development of *E. coli* bearing plasmids for enterotoxin by plasmid-free *E. coli* could be prevented, but this was in germ-free animals, and the *E. coli* was not able to attach on to the mucosa. In children the development of the pathogen Staphylococci has been prevented with non-pathogen lung Staphylococci.

**Question**

*From Dr. Viso (Alfort) to Dr. Ducluzeau*

Have you heard of any field experiments on calves for bacterial implantation at birth with commercially available products ?

**Answer**

The commercially available products currently used are generally composed of *Lactobacilli* and *Streptococci*.

No one has demonstrated that these bacterial species play a barrier role against some pathogenic bacteria in the digestive tract. So I think that these bacterial mixtures are ineffective.

**Comment**

*From Dr. Contrepois about the communication of Dr. Ducluzeau*

My first work with *E. coli* diarrhœa in calves, ten years ago, dealt with the implantation of bacteria in the young calf in order to inhibit *E. coli* proliferation. I never succeeded in obtaining a positive result. Now we know that the pathogenic process implies a proliferation of *E. coli* in the anterior part of the calf gut. In the jejunum of healthy calves, there is a low number of bacteria $10^5$-$10^6$/g in intestinal contents. So it is probably difficult to obtain a barrier effect in this part of the gut, as opposed to the caecum, for example, where the number of bacteria reaches $10^{10}$/g.