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SYMPOSIUM ON 'GUT MICROFLORA AND NUTRITION IN THE NON-RUMINANT'

The relationship between the host and its intestinal microflora

By R. W. Schaedler, *Department of Microbiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania* 19107, *USA*

During the past decade, there has been an increased interest in the microflora of the gut and its effects on the host organism. Several factors have contributed to this development. These include more sophisticated techniques for isolating and enumerating the anaerobic bacteria, the availability of germ-free and pathogen-free animals as experimental models, use of antibiotics with selective action, and better understanding of the metabolism of gut micro-organisms.

Much of the preliminary work has been done in animal models, and recent work in man indicates that similar situations exist in the two systems. Through the use of experimental models, it has been shown that the gastro-intestinal flora (1) affects the growth and development of the animal, (2) influences the nutritional requirements, (3) affects the morphogenesis of the gastro-intestinal tract, (4) modifies, through metabolic activity, both endogenous and exogenous substances presented to the gastro-intestinal tract and (5) plays an important role in preventing other 'foreign' organisms from becoming established in the gut.

One can now approach with greater confidence the symbiotic and parasitic aspects of the relationship between the gut flora and the host animal.

Intestinal flora, growth and nutrition

The growth of animals is closely correlated with their intestinal flora. There are several lines of evidence to support this. Giving low doses of broad spectrum antibiotics affords conventional animals a better gain in weight, whereas giving antibiotics has no effect on germ-free animals. Pathogen-free animals derived by Caesarean section and reared in a clean environment gain weight more rapidly than the conventional animals from which they are derived, as long as they are kept in a clean environment. That this is in a large part due to the intestinal flora was demonstrated by Dubos & Schaedler (1960). We showed that the clean, pathogen-free animals could be made to respond in the same way as conventional animals by contaminating them early in life with the faeces of conventional mice, or with pure cultures of coliform bacteria isolated from conventional animals.

The commonly studied aerobic bacteria, which are easily cultured from the faeces of most animal species, actually comprise only a small percentage of the total faecal flora of the pathogen-free mice. Studies which include both the anaerobic and aerobic organisms of the gut have given better insight into the problem.

The investigation of the development of the gastro-intestinal flora of pathogen-free mice offers several advantages since the experimental conditions are uniform and reproducible. As a newborn mouse begins to suckle, an intestinal flora develops.

The first bacteria to become established after birth are the lactobacilli and anaerobic streptococci. They increase very rapidly to 10^9 colony-forming units/g organ homogenate in the stomach and remain unchanged throughout the life-span of the mouse. Similar findings are seen in the small intestine, where these same bacteria are found, but at populations one-tenth of those found in the stomach.

In the large intestine the situation is far more complex, as depicted in Fig. 1.

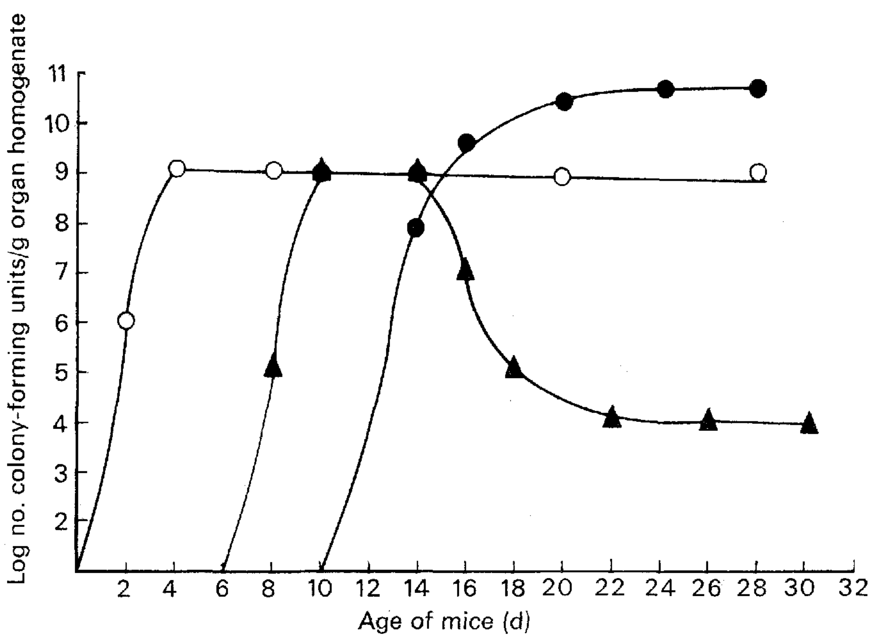

Fig. 1. The bacterial colonization of the large intestine of the mouse. ○——○, lactobacilli and anaerobic streptococci; ▲——▲, coliforms and enterococci; ●——●, obligately–anaerobic bacteria.

Lactobacilli and anaerobic streptococci appear by the 2nd day and persist for the life of the animal. Enterococci, slow lactose-fermenting (SLF) coliforms, *Escherichia coli*, or both, appear in the faeces on day 10 and proliferate to 10^9/g by day 12. Around the 16th day of life, however, their numbers have already begun to diminish drastically. This sudden decrease in the populations of coliforms and enterococci is accompanied by the appearance of an extremely heterogeneous population of obligately anaerobic bacteria, many of which are completely oxygen-intolerant, at the time when solid food is first ingested.

The most conspicuous bacteria of the faecal flora of weanling and adult mice are the so-called fusiform-like organisms which first appear in the large intestine on day 12. Within a week, these spindle-shaped (hence 'fusiform') bacteria outnumber all other members of the faecal flora by a ratio of at least 10 to 1, corresponding to populations of 10^{10}–10^{11}/g caecal homogenate. They are characterized as being predominantly Gram-negative, obligately-anaerobic rods with tapered ends. Some strains were supplied to Dr W. E. C. Moore at the Anaerobe Laboratory of the Virginia Polytechnic Institute in Blacksburg, Virginia. Members of the genera *Fusobacterium*, *Eubacterium* and *Succinomonas* were identified, but as was expected, some of the strains did not fit any known genus. Bacteroides are the next most populous members of the faecal microflora. They also make their appearance on the 14th day of life.

In the large intestine of the adult mouse, the bacteria present at the lowest concentration are the coliforms and enterococci. They are eventually reduced to numbers of 10^4–10^5/g faeces, thereby representing approximately 0·001% of the total faecal microflora of the adult pathogen-free mouse. In conventional laboratory mice, the aerobic microflora varies both qualitatively and quantitatively. Many more species of aerobic Gram-negative organisms are present and their numbers may reach up to 10^9/g faeces.

When groups of pathogen-free animals and conventional animals are fed on a purified diet (15C), containing 15% casein supplemented with cysteine as the sole source of protein, the clean, pathogen-free animals gain weight better than the conventional animals as shown in Fig. 2 by the solid line. If gluten is substituted as a sole source of protein in the purified diet, the clean, pathogen-free animals gain weight, whereas the conventional mice often will not gain weight at all or only very slightly (Dubos, Schaedler & Costello, 1967). Even more striking results are obtained if maize is used as a sole source of food. The pathogen-free animals can gain weight, whereas conventional laboratory mice will lose weight and often many die.

It is well known that wild field mice can exist on maize as a sole source of food. It is interesting that the faecal flora of the few wild mice which were studied was very similar to that of the pathogen-free animal rather than the conventional laboratory mouse. It appears that the conventional laboratory animal has acquired a flora from the artificial laboratory environment, which is superimposed on its indigenous flora. These added bacterial populations are propagated because of adverse conditions and bad animal husbandry.

Table 1 compares the effects of administering ampicillin, bacitracin and erythromycin in the drinking-water of mice for a period of 2 weeks. Ampicillin has a very drastic effect on the flora of mice, completely eliminating the fusiforms, bacteroides, and lactobacilli in the first few days and these organisms are not recovered as long as the animals are given the antibiotics. However, after about 7 d on the drug, enterococci and the coliform bacteria increase in number and become the predominant flora. This is remarkably similar to the effect of pencillin G as reported by Dubos, Schaedler & Stevens (1963) and Savage & McAllister (1971). Administration of

Table 1. *Log no. of organisms recovered from the caecums of mice which had received antimicrobial agents in their drinking-water. The solutions were given* ad lib. *throughout the study*

		Ampicillin (0·3 g/l) Day:				Bacitracin (100 000 units/l) Day:				Erythromycin (0·3 g/l) Day:			
	Controls	1	2	7	14	1	2	7	14	1	2	7	14
Fusiforms	10	0	0	0	0	0	0	0	10	10	10	10	10
Bacteroides	9	0	0	0	0	9	9	9	9	0	0	0	0
Lactobacilli	9	0	0	0	0	4	4	3	0	6	0	0	0
Enterococci	5	0	0	3	6	0	0	0	0	2	0	0	0
Coliforms	4	0	0	4	8	9	9	9	9	4	2	2	5

bacitracin results in a more selective action on the faecal flora. Interestingly enough, the fusiform organisms were depressed for a period of up to 2 weeks, but the number of bacteroides was unaffected. The coliform organisms which are resistant to this drug showed a tremendous increase compared with untreated controls, whereas the susceptible enterococci were completely suppressed. Erythromycin also had a very selective action on the faecal flora. The bacteroides were suppressed without any demonstrable effect on the fusiform organisms. Even though this drug does not have much activity against the aerobic Gram-negative organisms, there was no increase in their numbers.

By the use of various antimicrobial agents of different spectra, it has been demonstrated that the fusiform organisms in the gut of the animal are mainly responsible for the control of the Gram-negative aerobic organisms as well as the enterococci. Several points can be made. In the clean pathogen-free animals, administration of antimicrobial agents which drastically affect the anaerobic flora, especially the fusiform-shaped organisms, may result in increased proliferation of the common Gram-negative aerobes and enterococci. This is usually associated with a decrease in weight gain of the animals. On the other hand, when small doses of the broad spectrum antibiotics are administered in the drinking-water or food of the conventional animals there is a decrease in many of the common aerobic organisms present, but a minimum disturbance of the anaerobic flora. This is usually accompanied by an increase in weight. The effects of the antimicrobials are much more pronounced in animals fed on purified diets and especially on protein-deficient diets. The difference in response between pathogen-free and conventional animals is illustrated in Fig. 2.

The effect of the intestinal flora on morphogenesis and physiology of the gastro-intestinal tract

The anomalous appearance and function of the gut of germ-free rodents clearly indicates that in the conventional animal the intestinal flora has effects which are essential for the development and well-being of the animal.

In the germ-free animal, there is decreased peristalsis which results in longer transit time, a reduction in the thickness of the lamina propria of the small intestine with a decrease in numbers of plasma and inflammatory cells, a decrease in mucosal

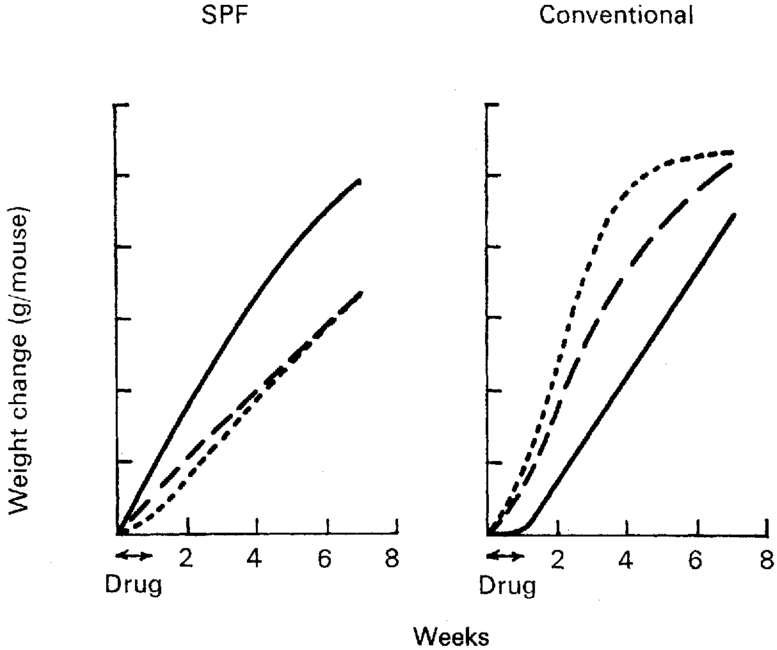

Fig. 2. Growth of specific pathogen-free (SPF) and conventional mice with and without the addition of antibiotics to the drinking-water. ———, control; — — —, penicillin; – – – – –, terramycin. Modified from Dubos, Schaedler & Costello (1963).

surface area, and a greatly reduced renewal rate of the intestinal epithelium. In addition, one of the most dramatic differences is a greatly enlarged caecum in the germ-free group. One explanation is that the caecal flora is responsible for inactivating an endogenous hypertensive enzyme. This kinin-releasing protease has now been characterized as trypsin and, when allowed to accumulate in the absence of rapidly metabolizing caecal flora, is thought to cause a relaxation of the smooth musculature with a subsequent enlargement of the caecum.

Animals may benefit nutritionally from harbouring an intestinal flora. Germ-free rats fed a diet free of vitamin K rapidly develop prolonged prothrombin times and haemorrhages, whereas conventional rats fed the same diet do not demonstrate these deficiency syndromes. Similar experiments conducted with diets deficient in the B vitamins showed that various bacteria, such as *Aerobacter*, *Alcaligenes*, and *Proteus*, were able to reverse some of the vitamin deficiencies when introduced into the gut of the animal. On the other hand some bacteria are thought to compete with the host for nutrients. This has been shown in germ-free chickens, colonized with the folic acid-requiring bacterium *Streptococcus faecalis*. Germ-free chickens placed on a diet low in folic acid and colonized with this organism grew at a decreased rate, whereas similarly fed chickens colonized with *Esch. coli*, a folic acid producing bacterium, were prevented from developing deficiencies. Thus, intestinal bacteria may compete with the host for vitamins which are in short supply and aggravate nutritional deficiences.

The intestinal flora also plays a role in the metabolism of bile acids. Various organisms isolated from the gut can deconjugate bile acids, for example they can dehydroxylate cholic acid to form deoxycholic acid. It has also been demonstrated that intestinal bacteria can remove the hydroxyl group in the 21 position of deoxycortisone, indicating that bacteria may play a role in the metabolism of steroids.

The role of the intestinal flora in preventing the establishment of other bacteria

It is very difficult to establish in the gut of a healthy adult mouse enteric pathogens such as *Vibrio cholera*, *Shigella*, *Salmonella* and staphylococci. In fact, it is difficult to establish non-indigenous bacteria, for example staphylococci, *Proteus*, and *Pseudomonas* in the gut of many species of animal. Freter (1956) and Bohnhoff & Miller (1962) found that by altering the normal flora with antibiotics, they could then establish many pathogenic species of organisms in the treated gut. In the germ-free animal all the organisms mentioned are readily established. The interaction of the various bacteria in the gut have been best studied in the germ-free animal.

Table 2 shows the results of associating germ-free mice with various indigenous bacteria; first, by giving two different strains of lactobacilli, one week later a culture of bacteroides, and after one more week, a culture of *Esch. coli* (SLF). Bacteriological tests carried out at weekly intervals revealed that the lactobacilli and the SLF organisms colonized the whole gastro-intestinal tract, whereas the bacteroides were

Table 2. *Number of bacterial colonies/g organ homogenate recovered* 1 *week and* 12 *weeks after giving multiple bacterial cultures to germ-free mice*

	Lactobacilli	Bacteroides	Slow lactose-fermenting *Escherichia coli*
Stomach	10^9	0	10^6
Small intestine	10^8	0	10^8
Large intestine	10^9	10^9	10^9

recovered only from the large intestine. The bacterial species behaved exactly as if they had been associated singly with germ-free animals. The only difference noted was that when the SLF organisms were mono-associated with germ-free mice, rapid lactose-fermenting mutants could be discovered at a high frequency. However, when the germ-free animals were first associated with lactobacilli and then the SLF organisms were introduced, none of the *Esch. coli* recovered from the mouse were rapid lactose-fermenters. It would appear that the lactobacilli utilized lactose as the available substrate and thereby prevented propagation of such mutants. It should also be emphasized, as illustrated in Table 2, that the coliform bacilli remained extremely numerous throughout the period of observation and that these organisms continued to colonize the stomach and small intestine.

In other experiments, *Staphylococcus aureus*, enterococci, *Proteus* and *Pseudomonas* were associated with germ-free mice. The numbers and distribution of these organisms were very similar to those of the SLF organisms depicted in Table 2. In every instance, when fresh faecal material from pathogen-free mice was introduced into the isolator containing germ-free animals associated with coliforms, enterococci or staphylococci, the numbers of aerobes rapidly decreased to 10^4/g faecal material, or less. Examination of faeces at this time revealed large numbers of the strict anaerobic, fusiform-like organisms. In other experiments, pure cultures of the fusiform organisms were introduced into isolators which contained animals mono-associated with either *Staph. aureus* or *Esch. coli*. In many instances, complex mixtures of these fusiform-like organisms caused a decrease of the *Esch. coli* or staphylococci to 10^4 or less. The indigenous lactobacilli, bacteroides and SLF *Esch. coli* did not give rise to any inflammatory response in the gut of these mono- or poly-associated animals. In fact, it was difficult to distinguish by histological examination of the large and small intestine between the germ-free and experimentally-associated animals. Although it is still difficult to enumerate the anaerobic flora by species, the evidence is extremely convincing that this main component of the gastro-intestinal flora is an important factor in the control of other organisms which may find their way into the gastro-intestinal tract.

Summary

There is an indigenous flora that is essential to the growth and development of the host and contributes to the host's well-being. The ideal flora will allow optimum weight gain, growth and development, and allows the host to be less demanding nutritionally. Furthermore, it allows for the morphological development of the gastro-intestinal tract without a large amount of inflammatory response. This basic microflora of the gut plays an important role in preventing other foreign organisms from becoming established. Alteration of this indigenous flora by diet, environment or antibiotics can be deleterious to the host.

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