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PHENOTYPIC EXPRESSIONS IN THE SMALL INTESTINE

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INTRODUCTION

Every man and animal is born germfree, i.e., without any microorganisms, and the colonisation of all surfaces such as the respiratory, urinary and alimentary tract starts immediately after birth. Initially, when space is not limited, bacteria with a high multiplication rate may dominate, but as the number of bacteria increases and accessible nutrient pools becomes limited, habitats will be filled up by more specialised bacterial species and the complexity of the flora increases.

When trying to investigate the composition of samples from any site the alimentary tract other than the mouth or lower part of large intestine from volunteers, it is difficult to obtain proper samples. Endoscopic sampling for microbiological evaluations has both advantages and disadvantages, and some investigators have also pointed on some difficulties in the sampling depending on from where it is taken in the stool specimens. Porcine faecal material was investigated with regard to microbes present in materials from the inner and the outer content, and differences were found due to atmospheric conditions. Another problem arises for instance due to storage and/or freezing and transport conditions from the sampling to analysis – these variables will also influence the final findings (*Rall* et al., 1970). Some of these problems can however be overcome today by the use of polymerase chain reaction and direct sequencing of 16S ribosomal DNA analysis of the flora. However, there are still limitations and the detection level is approximately the same as in previously well established microbiological evaluations. A complementary method is to evaluate what the flora has done, i.e., to evaluate the products – the outcome - of the crosstalk between the host and its microflora. Using this technique, substances produced in, e.g., the small intestine can be detected in faecal samples and thus reflect microbiological products from areas normally difficult to obtain samples from.

Comparisons of conventionally raised (Conv) organisms and germfree (GF) counterparts have revealed series of anatomic, biochemical, immunological and physiological phenotypes, collectively known as Microflora-Associated Characteristics or MACs. When the functionally active flora is absent, as in GF animals, healthy new-borns and sometimes in relation to antimicrobial treatment, a MAC is termed GAC (Germfree Animal Characteristic) (*Midtvedt* et al., 1985). Some phenotypic expressions are presented in Table 1. In the following, some of these phenotypic expressions, occurring in the small intestine, will be presented and discussed.

Table 1: Some anatomical structures, physiological and biochemical functions influenced by the microflora, and microorganisms involved (Modified from *Midtvedt*, 1999)

Parameter	MAC[1]	GAC[2]	Microorganism
Anatomical/Physiological			
Caecum size (rodents)	Normal	Enlarged	Partly known
Cell kinetics	Normal	Slower	Unknown
Colloid osmotic pressure	Normal	Increased	Unknown
Electro-potential Eh, mV	Low (<-100)	High (>-100)	Unknown
Intestinal wall	Thick	Thin	Unknown
Migration motor complexes	Normal	Fewer	Unknown
Osmolality	Normal	Reduced	Unknown
Oxygen tension	Low	High	Several species
Production of peptides	Normal	Altered	Unknown
Biochemical			
β-aspartylglycine	Absent	Present	Species in concert
β-glucuronidase	Low activity	High activity	Several species
Bile acid metabolism	Deconjugation	No deconjugation	Many species
	Dehydrogenation	No dehydrogenation	Many species
	Dehydroxylation	No dehydroxylation	A few species
Bilirubin metabolism	Deconjugation	Little deconjugation	Many species
	Urobilinogen	No urobilinogen	A few species
Cholesterol metabolism	Coprostanol	No coprostanol	A few species
Faecal tryptic activity	Little or absent	High activity	A few species
Intestinal gasses	Carbon dioxide	Some CO_2	Many species
	Hydrogen	No hydrogen	Some species
	Methane	No methane	A few species
Mucin	Degradation	No degradation	Several species
Short-chain fatty acids	Large amounts	Far less	Many species
	Several acids	Few acids	

[1] Microflora-Associated Characteristic; [2] Germfree Animal Characteristic.

CELL KINETICS

Since long time it has been assumed that the rate of crypt epithelial cell proliferation in the intestine represents a major defence mechanism against invading intestinal microorganisms. The cell renewal system involves proliferation of undifferentiated epithelial cells followed by differentiation and migration from the site of production to the functional site and finally elimination from the mucosa. Under normal conditions in mice, the cell turn over rate has been estimated to be about 10^8 cells per day (*Hageman* et al., 1970). By introducing strict standardisation techniques, we have been able to show that there are different phenotypic expressions in different compartments of the intestine in GF and Conv rats and mice with regard to age, gender and microbial status. In these studies, also diet and fasting time was standardised. Cell kinetic and morphological parameters reflecting the crosstalk between the host and its flora was investigated (*Banasaz* et al., 2000, 2001).

We found a great similarity between the mitotic index in young and old rats and mice, being high in the upper part of the small intestine and lower in the

colon, and we also found higher mitotic indexes in males as compared to females. A second similarity was that the crypt/villus ratio was on the same level throughout the small intestine. Thus, we found that in those areas, where the number of microbes was high, microbes triggered the mitotic index. We also found that, when establishing a microbial strain as a mono-contaminant, an immediate triggering of the mitotic index was seen, irrespectively of whether the mono-contaminant was a probiotic microbe (*Banasaz* et al., 2002), a pathogen or a commensal microbe (*Banasaz*, 2002).

When a toxin producing *Clostridium difficile* strain was mono-inoculated into young rats, there was initially an increase of the mitotic index. However, within some few days there was a marked decrease of the mitotic index and also occurrence of some epithelial border disruptions without leading to any disease or other signs of discomfort in the animals was seen. Lack of disease signs does not exclude that *Clostridium difficile* is a pathogenic microbe. By opening up for other microbes to cause disease, as we observed some few patchy morphological alterations in the intestinal mucosa of the rats, the disease signs could be caused by secondary infections. This study needs to be expanded to animals harbouring a normal flora.

INTESTINAL MOTILITY

It is well known that several microbial species may cause increased intestinal motility, expressed as cramps and diarrhoea, due to many different mechanisms. GF animals are known for having far less spontaneous muscular contractions than their Conv counterparts. Already in the sixties, a slower transit time in GF animals was reported (*Abrams* and *Bishop*, 1967; *Gustafsson* and *Norman*, 1969). Obviously, the intestinal flora plays an important role related to intestinal motility and transit time – some species influencing the small intestine (*Salmonella* spp.) and others the large intestine (*Shigella* spp.).

The microbes responsible for inducing motility, contractions and increased transit time, found under normal physiological conditions, are not known. However, intestinal movements are of a paramount importance for the regulatory and protective role of the microflora, and for the host (*Midtvedt*, 1989).

SHORT CHAIN FATTY ACIDS

The intestinal microflora ferments the dietary and endogenous large carbohydrates into mono- and di-saccharides in the small intestine and these appear to be the main contributors to the energy requirement in mammals after an anaerobic fermentation. The origin of intestinal short chain fatty acids has been substantiated in studies of GF and Conv rats and mice, and the faecal content of these acids are representing the net sum of production, absorption and secretion of the acids throughout the whole intestinal tract.

In the mouth of man, there are quite high amounts of these acids present, representing products of an anaerobic metabolism in the gingival pockets. In the stomach and upper small intestine, the amount of these acids is quite low

under normal, healthy conditions – in contrary to, e.g., patients with microbial small intestinal bacterial overgrowth. Findings from these patients indicate that there are overgrowth symptoms - the patients seem to have a colon-like flora in the lower small intestine, and the main part of these acids are produced by the altered flora in the jejunum (*Høverstad* et al., 1885). As an example can be mentioned that in 6 healthy volunteers, the short chain fatty acid content in saliva was quite high (2780-9940 µmol/l), decreased successively to 185-1470 µmol/l in jejunal juice, and the main part was acetic acid (approximately 85%), propionic acid accounted for almost 11%, and less than 2% of iso- and n-butyric and i-valeric acid (*Høverstad* et al., 1984). A very similar relative distribution was earlier found in the saliva, gastric juice and duodenal aspirates, however, essential different from the faeces content. This depends on the fact that, as the number of microbes increases in the lower small intestine and in the colon, also the short chain fatty acid content is altered in the composition, and totally 24-243 mmol/kg faeces – with significantly higher amounts in men as compared to women, have been found (*Siigur* et al., 1994).

INTESTINAL TRYPTIC ACTIVITY

Trypsin is chosen as a model substance for studying endogenous derived digestive enzymes. The precursor – trypsinogen - is excreted from the pancreas and activated in the upper part of the small intestine, mainly by brush border enzymes.

In total, faecal tryptic activity involves the net sum of processes such as secretion of trypsinogen from the pancreas, activation of the pro-enzyme in the small intestine by enterokinase and presence of host-, microbial-, and diet-derived compounds that inactivate or otherwise degrades the trypsin molecule during the passage through the intestine. In GF rats, tryptic activity is detected in the upper small intestine already at two days of age, and as the animals grow older, tryptic activity is detected in increasing amounts all the way down of the intestine and in faecal samples, where high amounts of the enzyme activity is detected. This in contrast to what has been found in materials from Conv animals, which are more or less devoid of tryptic activity in the lower intestine.

It is also shown that new-born children excrete faeces/meconium without any tryptic activity, although immunological studies detect the molecule – not yet activated (*Norin*, 1985). Thereafter, the enzyme activity increases successively during the first year of life followed by a decrease down to adult values some years later (*Norin* et al., 1985).

In Conv animals it is found that the enzymatic activity disappears mainly in the caecum. Only very seldom, a low level of tryptic activity is detected in the lower intestine and in faecal samples from rats and mice. It is found that the amount of this enzyme activity is variable depending on species investigated and on which diet the animals is given. Horses and pigs show the same low levels of faecal tryptic activity (*Collinder* et al., 2000, 2002), as found in e.g. rats, mice and man.

Obviously, intestinal microbes are responsible for the inactivation of trypsin, and at least one human strain of *Bacteroides distasonis* (*Ramare* et al., 1996) has been isolated and found ca-

pable to inactivate pancreas derived trypsin in both rats and mice. Previously, in Crohn´s disease patients, there were found high levels of tryptic activity in faecal samples as compared to samples from healthy volunteers (*van der Merwe* and *Mol*, 1982), and intestinal tryptic activity could possibly contribute to the pathological symptoms observed, mainly in the lower intestine of these patients. These microbes are also found to be influenced by several antimicrobial drugs, when given to rats (*Norin*, 1997).

BILIRUBIN AND UROBILINS

The bile pigments consist partly of bilirubin, a toxic and water insoluble end-product after catabolism of haemoglobin and some other haem-containing substances. The reduction of bilirubin to urobilins by intestinal microbes represents one natural detoxification process of toxic intestinal substances such as xenobiotics, drugs, hormones and certain dyes. Bilirubin as well as other toxic substances is conjugated in the liver with glucuronate to a less toxic and water-soluble molecule, which are secreted with the bile into the intestine.

In the intestine, the bilirubin conjugates are de-conjugated by ß-glucuronidases and further transformed to series of metabolites, most often termed urobilins. Most of the ß-glucuronidases are derived from the microbes, only a minor part is produced by the host. This detoxification process will be discussed in a deeper setting later during this meeting. Enhancement of microbial conversion of bilirubin to urobilins decreases the intestinal concentration of bilirubin, which is a potential risk factor for hyperbilirubinaemia, which can lead to extrapyramidal disturbances, hearing loss, delay in motor development and less often, also to intellectual deficits (*Saxerholt*, 1990; *Vitek* et al., 2000).

CONCLUDING REMARKS

The normal intestinal flora plays an important regulatory and protective role in all organisms, but external disturbances could be harmful for the host and its flora. It is, e.g., known that antimicrobial treatments causing marked alterations of the flora-composition could induce altered intestinal functions. Supplementation of the intestinal microflora with live microbial species, used for many years to protect and maintain the balance in the intestine, could eventually also be a potential risk factor. Introduction of live microbes when e.g., the intestinal flora is still not fully settled or otherwise strongly disturbed, could alter the succession of the establishment of the normal flora. One could thus speculate that, when the intestinal flora is immature and continuously developing, an altered succession of the "normal" establishment pattern of the flora could cause unexpected consequences related to functions both in the small and in the large intestine.

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