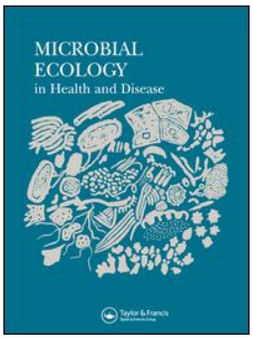

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Gastrointestinal Host–Microbial Interactions in Mammals and Fish: Comparative Studies in Man, Mice, Rats, Pigs, Horses, Cows, Elk, Reindeer, Salmon and Cod

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The gastrointestinal tract of all animals is colonized with a vast, complex and dynamic consortium of microorganisms. In spite of recent progress in molecular microbiology, this complexity makes a proper qualitative and quantitative evaluation difficult and expensive to perform. In the present review we discuss the results obtained from applying a functional concept for studying intestinal host–microbial interactions in eight mammalian species and two species of fish. We show that microbial interactions with endogenous derived substrates such as cholesterol, bilirubin, mucin and trypsin occur in all species. Exogenous compounds (e.g. carbohydrate, protein, fibre) are also acted upon by the intestinal microflora. From an evolutionary point of view, dietary differences have led to a compartmentalized variability in microbial functions. Taken together, the results in this review underscore the importance of understanding precisely how gastrointestinal metabolism serves to establish a symbiotic cross-talk between animals and their gastrointestinal flora. *Key words:* mammals, fish, microflora-associated characteristics, short-chain fatty acids, cholesterol.

INTRODUCTION

Animals (including humans) are born germ-free (GF), but soon after birth they are colonized with microorganisms. Then, series of life-long interactions or cross-talk between the host and its microflora begin. In this review we will discuss the microflora of the gastrointestinal (GI) tract and how it may interact with the host. We will focus on recent insights as to how the microorganisms help the host in degrading dietary and endogenous compounds.

THE MICROFLORA

The digestive tract of adult animals and humans harbours a microbial flora, characterized by its diversity, density and complexity. The specific composition of the flora will partly be influenced by environmental factors such as diet and temperature, and partly by a long series of endogenous factors, such as pH, redox potential and retention time for digesta (1, 2). Normally – at least in all mammals so far investigated – microorganisms constituting the flora in the digestive tract outnumber the cells in the body of the host by at least one log. Most often (when it is known) the intestinal microflora is constituted of hundreds of species belonging to many different families and genera. This complexity makes a proper qualitative and quantitative evaluation difficult, time-consuming and expensive to perform, even by modern, molecular methods.

Another approach is to look at the microflora from a functional point of view. The microflora is involved in a multitude of biochemical reactions and can collectively be thought of as a metabolically active 'organ'. It is well recognized that this metabolic entity plays a critical role in the nutritional status and well-being of the host.

Comparisons of conventionally raised animals with their GF counterparts have revealed a series of anatomic, biochemical, immunological and physiological phenotypes collectively known as microflora-associated characteristics (MACs). When a functional microflora is absent, as in GF animals, newborns and sometimes following intake of antimicrobial agents, the values of the various parameters are collectively termed germ-free animal characteristics (GACs) (1, 3).

Over the years we have applied this MAC/GAC concept when studying intestinal host–microbe interactions in different animal species undergoing various changes, such as variations in diet (4), exposure to antibiotics (5, 6), etc.

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This review discusses the outcome of applying the MAC concept to animals with major differences, either in the structure of the digestive tract or in their way of living (environmental or dietary differences).

DIGESTIVE TRACT AND DIET

Animals can be classified, according to the diet in their natural state, as carnivores (meat-eaters), herbivores (plant-eaters) or omnivores (all-eaters). On the other hand, according to their body temperature, animals can be classified as homeothermic (endothermic), such as mammals and birds, or poikilothermic (ectothermic), such as fish, amphibians and most reptiles. Man and pigs are typical omnivores, whereas ruminants are herbivores. In this review fish are exemplified by salmon (*Salmo gairdneri*) and cod (*Gadus morhua*), both carnivores.

The herbivores make up $>90\%$ of the total mammalian population (7). They rely, to a large extent, on their intestinal microflora for degradation and digestion of food. No animal species produce enzymes for digestion of structural carbohydrates such as cellulose, hemicellulose and pectins. These compounds are the major constituents of all plants and are therefore regarded as the major nutritional factors in the diet of all herbivores.

The dependency of herbivores on their intestinal microflora is due to their phylogenetic background and alterations in the anatomy of the digestive tract. In the main, Mother Nature has come up with two strategies: foregut fermentation and hindgut fermentation. In foregut fermenters, the fermenting chambers are placed before the small intestine. That means the first part of the stomach, orally to the part producing gastric juice. Ruminants (*Ruminantia*) have their fermentation in the rumen and reticulum, camels and llamas in compartments one and two. Other foregut fermenters have a more or less sacculated tubular stomach. In hindgut fermenters, the fermenting chamber is placed after the small intestine, in the form of the caecum and/or the first part of the colon, which are enlarged.

Bacteria constitute most of the biomass in the GI tract, but fungi and protozoa might be present. Especially in ruminants, fungi and protozoa contribute to cross-talk between microorganisms and the host.

In foregut fermenters, food is attacked by microorganisms before the enzymatic digestion in the lower part of the stomach and the small intestine. In hindgut fermenters, as in carnivores and omnivores, an enzymatic digestion precedes the microbial fermentation in the large intestine. In foregut fermenters the host easily digests microbial proteins and utilizes the microbial vitamins when microorganisms pass with food residues along the digestive tract (7). A disadvantage of this system is that fermentation of easily digestible carbohydrates, such as starch and sugars, results in loss of some energy (e.g. production and discharge of methane). The hindgut fermenters utilize some types of starch and sugars without energy loss in the small intestine, but in most cases they are less efficient in utilizing microbial protein and vitamins. However, many small herbivores can utilize microbial protein by means of caecotrophy, a selective ingestion of high-nutrient faeces derived from caecal contents (2, 8).

The basal energy need is proportional to body mass (W) raised to 0.75 ($W^{0.75}$), by an allometric '$\frac{3}{4}$ power law' (9, 10). Thus, small animals have a much higher energy need per unit body mass than large ones (11). Small animals living on coarse food do not have space enough for a large fermentation chamber, and therefore the digesta has to move rapidly through the digestive tract. To be able to retain functionally active microorganisms and the most easily digestible food components, these animals (most rodents and all lagomorphs, i.e. rabbits, hares, etc.) need anatomical and functional specializations to combine enzymatic digestion in the foregut with microbial fermentation in the large intestine. This means they must be hindgut fermenters. For instance, the anatomy of the digestive tract of mice (*Mus musculus*) and rats (*Rattus norvegicus*) is very similar (Fig. 1) but functionally a mouse of about 30 g needs nearly twice as much energy per unit body mass as a 300 g rat. Both species ferment food residues in the caecum and they have the capacity to trap bacteria in mucus secreted in the most proximal part of the colon and transport them in a retrograde direction back into the caecum (7, 12). The surplus of microorganisms is harvested by caecotrophy and microbial amino acids and vitamins are utilized by the rodent after digestion in the stomach and the small intestine. The same mechanism also occurs in GF rats and mice, suggesting that the behaviour is inherent (13).

Humans (see Fig. 1) also get a proportion of their maintenance energy ($<10\%$) as a result of microbial fermentation in the most proximal part of the colon (14, 15). Compared with pigs (*Sus domesticus*) (Fig. 2) man has a small and short caecum and colon and adult pigs may get as much as 30% of their maintenance energy from microbial fermentation in the caecum and proximal part of the colon when eating roughage (2, 16).

The horse (*Equus caballus*) is a grazer utilizing three consecutive chambers for fermentation, the caecum, the ventral large colon and the dorsal large colon (see Fig. 2). They have a less efficient fermentation than ruminants. In contrast to cattle, they can survive on very coarse food, provided that the food volume is large, as horses increase their food intake when the energy density is low. Cattle decrease their food intake under the same condition due to a prolonged retention of digesta. Horses have an ability to retain microorganisms at the end of the large colon when eating very coarse food (8, 17). This may result in a more efficient fermentation.

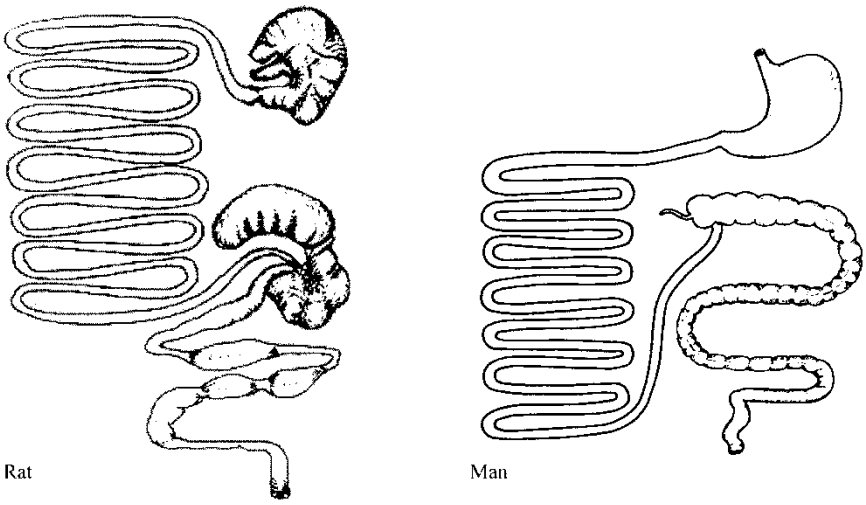

Fig. 1. The gastrointestinal tract of two omnivores: rat, caecum fermenter; man, colon fermenter (modified from ref. (79)).

The most efficient fermentation occurs in ruminants and in camelides (camels, llamas) due to mechanisms that delay the food residues in the stomach. Apart from the large fermentation compartment of the stomach they also have fermentation in the hindgut, but to a lesser extent.

Groups of ruminant species differ in their feeding behaviour. Grazers such as cattle (*Bos taurus*) (Fig. 2) prefer grass as food and retain it for a very long time in the rumen and therefore they have a very efficient fermentation. Elks (*Alces alces*) and reindeers (*Rangifer tarandus*) are classified as browsers. They select more easily fermentable food components and retain them for a shorter period of time in the rumen (18).

The intestinal flora of fish (Fig. 3) and other poikilothermic animals has another composition and less stability than the flora in homeothermic animals, and its role is still partly unclear. It is claimed that the intestinal flora in fish mostly reflects the feeding and drinking habits of the animal and is therefore a function of the surroundings (19–21). Temperature and drinking habit might influence their flora (11, 22). The body fluid of freshwater teleosts is osmotically more concentrated than their surroundings, therefore they undergo a steady osmotic influx of water, mainly through the gills. The body liquid of marine teleosts, on the other hand, is osmotically more dilute than the water in which the animals live, therefore they constantly lose water, primarily through the gills. To compensate for water loss, the marine teleosts drink substantial amounts of sea water (11). Since all fish species are poikilothermic, their flora reflects environmental temperature. Additionally, drinking habit in marine fish may also have a marked influence upon the intestinal flora, making the GI tract most suitable for aerobic microbes. The high surrounding bacterial load may also influence the functions of the flora.

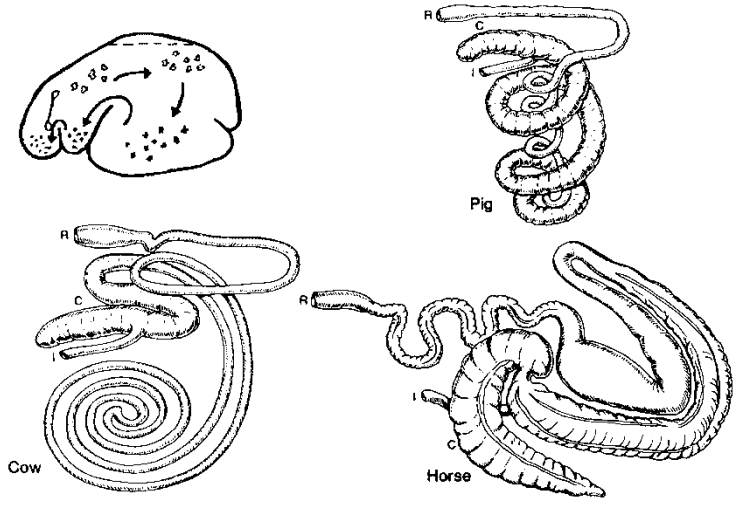

Fig. 2. Some details of the gastrointestinal tract in three domestic animals. (a) Rumen in cow; (b) large intestine in pig; (c) large intestine in cow; (d) large intestine in horse (modified from ref. (92)).

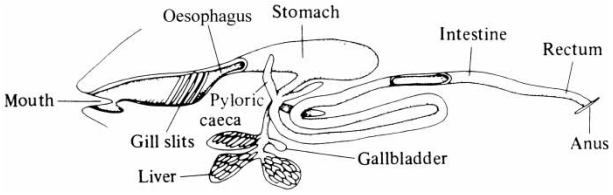

Fig. 3. Schematic drawing of the digestive system in teleosts (modified from from ref. (93)).

FUNCTIONAL STUDIES IN MAMMALS AND FISH

Conversion of cholesterol to coprostanol

The cholesterol present in intestinal content derives from both endogenous and exogenous sources. The endogenous cholesterol is the result of synthesis mainly occurring in the liver and the small intestine. The exogenous cholesterol derives from food of animal origin. The intestinal cholesterol may be absorbed, or may undergo microbial conversion to different metabolites, of which unabsorbable coprostanol is by far the most dominant (23).

Conversion of bilirubin to urobilins

Bilirubin, a degradation product of haemoglobin, is conjugated in the liver and then excreted with the bile into the intestine. In the intestine the bilirubin is deconjugated and also converted into a series of breakdown products, usually called urobilins. Most of these transformations are carried out by microbial enzymes (24–27).

Degradation of mucin

The mucosal surfaces of the body are covered by a highly hydrated gel, the mucus. The major part of mucus is water, but the gel matrix is formed by large and complex glycoproteins, the mucin (28). The mucin is successively degraded into monosaccharides by microbial enzymes, and thereby represents an important endogenous source of carbon and energy for the intestinal flora.

Inactivation of tryptic activity

As a response to food intake, inactive trypsinogen is secreted by the pancreas into the small intestine to deal with and break down ingested proteins. To prevent intrapancreatic activation of trypsinogen, which might lead to auto-digestion, trypsin inactivators are secreted together with trypsinogen. In the intestine, trypsinogen is activated to trypsin either by enterokinase or by already formed trypsin. Trypsin and/or the trypsin inactivators are then successively inactivated/degraded by microbial enzymes and exogenous products (29, 30).

Degradation of β-aspartylglycine (β-asp)

It is generally assumed that the presence of certain dipeptides, especially β-asp, in faeces of conventional (CV) mammals, including man, indicates that the normal intestinal microbial ecosystems are seriously altered. Degradation of β-asp has therefore been investigated as a so-called colonization resistance factor, namely a barrier against the establishment of opportunist pathogenic microorganisms (31, 32).

Formation of short-chain fatty acids (SCFAs)

SCFAs are the main end products of anaerobic microbial metabolism of carbohydrates and proteins in the GI tract. More than 90% of SCFAs produced are rapidly absorbed unionized, during the passage of ingesta through the GI tract (33). The absorption rates of the unionized acids are, according to their lipid solubility, butyric > propionic > acetic (34). Almost all of the SCFAs remaining in digesta are ionized, acting as dominant anions in the large intestine and faeces (2). They are claimed to be implicated in several physiological and clinical conditions (Box 1).

ANIMAL SUBJECTS INVESTIGATED

Mice

A total of 14 GF and 19 CV NMRI-KI mice (male and female, average age 3 months) was used. The GF animals were born and reared at the Department of Medical Microbial Ecology, Karolinska Institutet, Stockholm, in lightweight stainless steel isolators. The CV mice were reared in an ordinary animal room with artificial light between 6 am and 6 pm, temperature $24^\circ \pm 2^\circ$C and humidity $55 \pm 10\%$, at the same department. All the animals were fed an autoclaved rodent diet, R36 (Lactamin, Sweden) and had free access to water (35).

Rats

A total of 15 GF and 36 CV AGUS rats (male and female, average age 5 months) was used. The animals were reared, housed and fed in similar conditions as described for mice and at the same department.

Pigs

Faecal samples from 15 GF minipigs (Miniature Minnesota Pigs; 22 days old) were used. They were reared at the Department of Immunology and Gnotobiology, Novy Hrádek, Czech Republic. The animals were delivered by caesarean section and housed in sterile plastic isolators (36). The GF minipigs were foddered *ad libitum* with full-cream condensed cow's milk that had been diluted to 16% dry matter (DM) and then autoclaved for 10 min at 124°C. The formula contained 3.6 g fat, 3.3 g protein and 4.7 g saccharides per 100 g. The vitamins provided in the diet were: vitamin A 1000 IU, vitamin D_2 200 IU and vitamin K_2 10 mg/1000 ml (37).

Eleven Swedish Yorkshire sows were used as CV pigs. The sows were born and raised at the Pig Research Herd,

Statement: Short-chain fatty acids (SCFAs) are normally produced in high amounts by the intestinal flora; they are partly absorbed and partly excreted in faeces

Mechanism behind possible consequences:
- Biochemical: SCFAs are involved in several metabolic pathways
- Immunological: Uncertain consequences

Place:		Locally:	In the intestinal lumen
			At the mucosa surface
			Within the mucosa cells
		Distant:	In the liver, pancreas, brain, etc.
Form:	Direct:	Locally:	Main anions in intestinal content
			Growth promotion of some microbes
			Growth suppression of others
			Growth regulation of mucosa cells
		Distant:	Energy supply to the general metabolism
	Indirect:	Locally:	'Promoted' microbes produce suppressive bacteriocins
			Direct suppression provides niches for other microbes to grow
		Distant:	Metabolic alternations act on production of insulin, etc.
Consequence:	Physiological:		Locally the SCFAs are parts of direct/indirect regulatory mechanisms for water and electrolyte absorption; the net effect is antidiarrhoeic, involved in regulation of carbohydrate metabolism, etc.
	Pathophysiological:		Involved in hepatic coma. Probably involved in colonic cancer, ulcerative and pseudomembraneous colitis, etc.

Box 1. A consequence analysis of one microflora-associated characteristic.

Swedish University of Agricultural Sciences, Uppsala, following the recommendations for fattening pigs in Sweden, and fed on general sow fodder (38).

Humans

Ninety-three volunteers from Queen Margaret College in Edinburgh, Scotland, UK participated in this study. The mean age was 25.4 years (range 18–58). All volunteers were within $\pm 20\%$ of their ideal weight according to the 1983 Metropolitan Life Insurance Company tables and were in good health as determined by past medical history, physical examination and screening laboratory tests. The average daily dietary fibre intake before the study, as assessed by a 5-day meal diary and food frequency chart, was 18.9 g (range 6–29). Antibiotics had not been taken for a period of at least 6 weeks before the study. The protocol was approved by the Ethics Committee of the Western General Hospital in Edinburgh, Scotland, UK (39).

Horses

Faecal samples were collected from 27 clinically healthy adult horses (5 thoroughbreds, 22 standardbreds; weighing 410–570 kg) at health examination, Mälaren Equine Clinic, Sigtuna, Sweden. They were all fed on their habitual home feed. All samples were taken manually from the rectum.

Cows

Three rumen-cannulated cows in late lactation, at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, were used. The cows were housed in a tie-up stanchion barn and milked twice a day. They received 7.5 kg DM of high quality (measurable energy >10.8 MJ per kg DM) grass silage and were fed 9.3 kg standard concentrate feed per day. The concentrates were partitioned and given four times a day and the silage was divided into two portions per day. Six samples were taken from the rumen and six from the rectum of each cow on different occasions; a total of 18 samples from each compartment. All faecal samples were taken manually from the rectum.

Elks

Faecal samples were obtained from eight healthy adult elks (male and female; weighing 300–500 kg) put to death during elk-hunting in October 2000 in Hälsingland, Sweden.

Reindeers

Faecal samples were obtained from 25 adult healthy reindeers (*Rangifer tarandus*) (male and female; weighing 60–80 kg) during the slaughter period in early January 2001 in Arvidsjaur, Sweden.

Fish

A total of 68 samples was collected from intestinal contents of salmon at eight different freshwater farms. A total of 21 samples was collected from intestinal contents of cod caught in a sub-arctic region, Lofoten, Norway. All samples were investigated for four MAC functions.

All faecal samples were frozen soon after collection and stored at -20°C until they were analysed for MACs.

The methods for testing are shown in Table I, as described by Collinder et al. (40). A flow chart of the methods is shown in Fig. 4.

RESULTS AND DISCUSSION

Conversion of cholesterol to coprostanol

As is evident from Table II, coprostanol was produced in all mammalian species, but it was never found in GF animals. The results will be discussed from (i) compartmentalization and (ii) functional points of view.

(i) Conversion of cholesterol to coprostanol is a function carried out by a few strictly anaerobic strains (41, 42). In rats and man it is claimed to take place mainly in the caecum (43). Previously, we have found a small amount of coprostanol in small intestinal content from some horses (44), indicating an anaerobic condition in that area. In an earlier study it was found that rumen fluid from sheep contains microbes capable of transforming cholesterol to coprostanol *in vitro* (45). Our results show that this conversion also takes place *in vivo* in the rumen of cows.

(ii) From a metabolic or functional point of view, conversion of cholesterol to coprostanol can be looked

upon as a sharp 'microbial intestinal knife', influencing the normal enterohepatic circulation of cholesterol (23). Cholesterol is a part of cell membranes in animals and all animals have a substantial endogenous production of cholesterol. This 'knife' represents one of several regulatory mechanisms for ensuring a stable – or consistent – cholesterol metabolism. On the other hand, faecal excretion of coprostanol represents a metabolic loss for the animal itself. Following these assumptions, faecal excretion of coprostanol should tend to be low in animals fed on plants only. Our results in elks and reindeers fit in very well with this suggestion (Table II). It should also be mentioned that the mice and rats in our studies received no dietary animal ingredients. The comparatively low value in mice may be related to the rapid intestinal transit time in small mammals, together with simultaneous depressed metabolism of ingesta.

Coprostanol has previously been reported to occur as early as 2–3 weeks after birth in piglets, with significantly higher concentration before weaning than after (38). However, this high excretion of coprostanol might be related to coprophagy from sow's faeces, which suckling piglets usually perform (46).

Fish drink generally little or no water when in freshwater, since it diffuses inward across the gills. On the contrary, seawater fish have water loss, which must be countered by water intake through drinking (11, 47). Such intake of sea water tends to produce more aerobic conditions to harbour cholesterol-converting species. Interestingly, in general the flora of marine fish has been described to contain aerobes such as *Vibrio* and *Pseudomonas* while freshwater fish are colonized by obligate anaerobes like *Bacteroides* and *Eubacterium* (48, 49). In conformity with that, cholesterol conversion was only found in some freshwater farmed salmon.

As shown in Table II, individuals with no conversion of cholesterol to coprostanol were only found in a few humans. Our data fit in with previous data from Norway (50, 51), Finland (52) and USA (53) showing that one in five adult healthy individuals might be non-excreters or low-level excreters of coprostanol. In a previous prospective long-term study in infants, this function was established from 6 months of age. We hypothesized that a genetically determined receptor determines *whether*, and an environmental receptor modulation determines *when*, cholesterol-converting flora will be established (54). So far, however, the nature of this receptor(s) is still unknown.

Additionally, it should be mentioned that this function is targeted by several antimicrobial drugs (5, 6). Taken together, the results indicate that antibiotics with an anaerobic Gram-positive profile have the most profound influence on this parameter.

Further studies are required to determine whether individual or antibiotic-induced alterations in this parameter will or will not influence other aspects of cholesterol metabolism, such as serum cholesterol levels and development of atheromatous arterial disease.

Conversion of bilirubin to urobilins

The absence of urobilins in GF animals confirms this as a GAC. The presence of urobilins in CV cohorts of several species shows that this function most likely is generally present in all mammals. Just a few microorganisms have the capability to perform this function (24–26, 55).

Since bilirubin is a breakdown product of haemoglobin, each mammalian species will have a relatively fixed bile excretion of bilirubin. The obvious lower value in mice compared with rats may, in the same way as coprostanol, reflect an increased intake, a faster intestinal passage and a depressed metabolism of ingesta.

The pattern for establishment of this function may differ from species to species (38, 56). The high concentration of urobilins in young piglets probably reflects neonatal high excretion of fetal bilirubin into the GI tract, together with

Table I

Microflora-associated characteristics (MACs) and corresponding germ-free animal characteristics (GACs) used as parameters

Parameters	MAC value	GAC value	Interactive field	Microbes involved	Methods for detection
Conversion of cholesterol to coprostanol	Coprostanol present	No coprostanol	Enterohepatic circulation	Few *Eubacterium* species	Gas chromatography
Conversion of bilirubin to urobilins	Urobilins present	No urobilins	Enterohepatic circulation	Few species *Bacteroides* and *Clostridium*	Spectrophotometry
Degradation of mucin	Mucin degraded	Not degraded	Intestinal mucosa	Several species	Electrophoresis
Inactivation of trypsin	Low or no activity	High activity	Pancreas	*Bacteroides distasonis*	Spectrophotometry
Degradation of β-aspartylglycine	β-aspartylglycine degraded	Not degraded	Dietary compounds	Mostly unknown	Electrophoresis
Formation of SCFAs	High amount of SCFAs, several acids	Low amount of SCFAs mainly acetic acid	Exogenous/endogenous compounds	Several species	Gas chromatography

SCFAs, Short chain fatty acids.

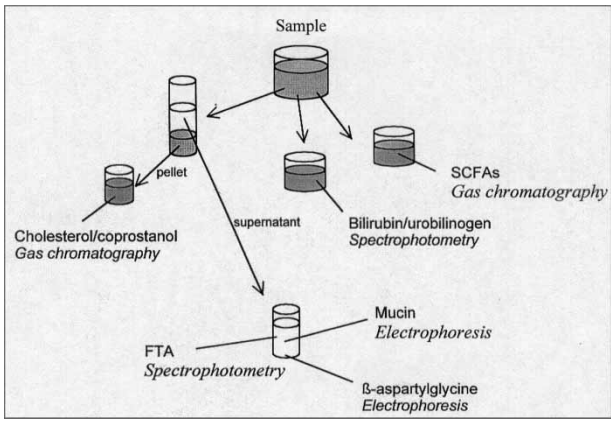

Fig. 4. Flow chart of investigation. The methods are described in detail in ref. (40).

additionally converting microbes and urobilins by ingestion of sow's faeces (46). When pigs grow up their faecal urobilins decrease (38). The low concentrations of urobilins in adult pigs might also be due to large stool mass diluting the constant end product of haemoglobin degradation – urobilins. In children this function is established within 6 months of age, although the values are considerably lower than in adults (56). The relatively high concentration of urobilins in humans might depend on a less diluted stool mass in humans than in the other mammals.

In the main, herbivores except reindeers show low faecal excretion of urobilins, probably due to fibre-rich diets. The high excretion in reindeers may partly be a result of the intense running they often perform in an enclosed collecting field before slaughter, mobilizing blood reserves from the spleen and high concentration circulating haemoglobin (57). Their sparse diet, dominated by lichen and woody plants (18), should also increase the faecal concentration of urobilins in the winter season. This parameter has been found to be higher in sport horses than in horses at rest, probably because of lower intake of fibre (40). Additionally, as circulating haemoglobin is higher in horses in training, more bilirubin should be available for reduction to urobilins than in horses without training (57).

As for the cholesterol parameter, conversion of bilirubin to urobilins is affected by the intake of antimicrobial agents that are effective against anaerobic Gram-positive bacteria (58).

Degradation of mucin

Mucin is not degraded in the GF animals, which excrete large amounts of mucin with their faeces, while degradation occurred in all CV adult mammals investigated (Table II). In GF rodents and lagomorphs (e.g. rabbits), the caecum becomes grossly enlarged containing large amounts of mucus, up to 30% of total body weight (59). This phenomenon depends partly on a mechanism to trap microorganisms and transporting mucus retrograde from colon to caecum, and partly on a general reduced motility in the large intestine (8, 60, 61). Both mechanisms tend to give a reduced excretion of mucus and consequently a passive dilation of the large intestine, especially the caecum.

Some microbial strains have been shown to be involved in degradation of mucin, by production of various glycosidases and peptidases (62). However, one strictly anaerobic *Peptostreptococcus* strain has been shown to degrade mucin totally with its peptidases (63).

Degradation of mucin in mammals increases with age until their flora has matured. Establishment of this function differs between piglets born outdoors and those born indoors, probably due to environmental factors (38). In infants, microbial degradation of mucin begins within the first 6 months of age and is completed during the second year of life. The priming of this function has also been found to be different between breast-fed and formula-fed children, probably owing to diet and environmental factors (56).

The presence of small amounts of mucin in the faeces of a few elks and reindeers (see Table II) might be due to a reduced breakdown of locally produced mucin covering the faecal pellets.

Degradation of mucin seems to be performed in all mammals, reflecting microbial cross-talk with the host. Complex dietary carbohydrates are generally assumed to be exogenous substrates for production of SCFAs, and mucin is a major endogenous source for this microbial production. Thus, complete degradation of mucin gives energy to the host. There is also growing evidence that mucin may be relevant in the pathophysiology of some intestinal diseases, such as ulcerative colitis, Crohn's disease, gastric and duodenal ulceration and colon adenocarcinoma (64, 65).

In samples from fish, all carbohydrate groups in mucin were degraded, but proteins were not degraded (Table III). This might be due to either an absence of microbe(s) able to degrade or an oxygen tension that is too high for microbial activity.

As for the cholesterol parameter, degradation of mucin is affected by the intake of antimicrobial agents that are effective against anaerobic Gram-positive bacteria (66).

Inactivation of tryptic activity

Trypsin is secreted by the pancreas in an inactive form, trypsinogen. It is activated in the intestine by the enzyme enterokinase, which is secreted by glands in the intestinal wall. Trypsinogen is also activated by active trypsin (i.e. trypsinogen is activated more rapidly as more trypsin is formed). This is known as 'autocatalytic activation' (11).

Faeces from GF animals contain large amounts of faecal tryptic activity (FTA), whereas far less is found in their CV counterparts (Table II). This important proteolytic enzyme is active mainly in the small intestine of CV adult mammals (29). So far, however, only one intestinal bacterial strain has been found to be responsible for inactivation of trypsin (67).

Table II

Microflora-associated characteristics in faeces from some omnivores and herbivores, and in rumen of cows; in mice, rats and pigs germ-free (GF) and conventional (CV) counterparts were investigated[a]

	Omnivores							Herbivores					
	Caecum fermenters				Colon fermenters			Colon	Rumen fermenters				
Animals (*n*)	GF mice (14)	CV mice (19)	GF rats (15)	CV rats (36)	GF pigs (15)	CV pigs (11)	Humans (93)	Horses (27)	Cows[b] (18)		Elks (8)	Reindeers (25)	
									Rumen	Faeces			
Coprostanol (%)	0	21	0	39	0	48	65	40	37	57	39	24	
		8–52		16–69		35–70	0–100	20–65	18–67	46–78	27–48	15–43	
Urobilins (mmol/kg)	0	0.19	0	0.27	NT	0.06	1	0.07	NT	0.08	0.07	0.31	
		0.12–0.26		0.17–0.36		0–0.45	0.3–2.4	0–0.09		0.06–0.11	0–0.09	0.08–0.83	
Mucin degradation (%)	0	100	0	100	NT	100	99	100	100	100	89	100	
							94–100		78–100		78–100	56–100	
FTA (mg/kg)	1014	78	1390	0	797	21	37	22	NT	10	0	9	
	696–1398	0–306	1250–1550	0–75	459–1058	6–27	0–398	0–63		0–21	0–21	0–126	
β-aspartylglycine degradation (%)	0	100	0	100	0	100	100	100	100	100	100	100	

[a]Values presented are medians, minimum–maximum; coprostanol is given as percentage of cholesterol plus coprostanol; degradation of mucin and of β-aspartylglycine respectively, graded according to percentage. [b]Number of specimens investigated. NT, not tested.

Conventionally raised animals require 30% less calory intake to maintain their body weight than their GF counterparts (68). It seems reasonable to assume that the high excretion of FTA found in GF animals represents a loss of energy from the host.

High intake of proteins stimulates secretion of trypsinogen, adjusted to deal with ingested proteins (29, 69). Differences in FTA values between species (Table II) might originate similarly from different diets (29). The comparatively high value in humans fits in with their relatively high intake of proteins. The high faecal value in mice may be due to their high intake and rapid intestinal transit time of ingesta.

The physiology of fish demands a diet containing a great deal of protein to survive (70); this is as much as two-to-four times more than mammals need. Thus, the pancreatic output of trypsinogen in fish should be stimulated. The generally higher level of FTA in carnivorous teleosts than in herbivorous and omnivorous mammals corresponds well with that (see Table III). Carnivorous mammals should also have higher levels of FTA than herbivores and omnivores, since they have high demands for protein and amino acids.

These findings collectively highlight some differences between fish and mammals regarding intestinal functions. Whether and to what extent the FTA values in fish are influenced by diet and environment should be further elucidated.

Antimicrobial drugs given to laboratory rats influence FTA (71, 72), but no significant effects have been found in humans (73), horses (44) or pigs (74). The difference between various animal species may result from a lower environmental load of trypsin-degrading microbes in cage-raised laboratory rats compared with more 'free-living' macro-organisms, such as man, horses or pigs (23). In our studies, FTA values in salmon treated with antibiotics were significantly higher than in untreated salmon and cod (Table III). Obviously, microbes inactivating trypsins are present in fish as in mammals.

Degradation of β-aspartylglycine

Degradation of β-asp is the least known parameter discussed in this review. Biochemical background for the presence of β-asp in faeces may be that dietary proteins are the main targets of intestinal proteolytic enzymes. Biochemically, β-asp is a member of a group of β-carboxyl dipeptides formed in the intestinal tract, when dietary proteins are broken down by host-derived proteolytic enzymes (23). The β-carboxyl peptide bonds are thought to be broken down only by proteases derived from microbes. This has been substantiated by the finding that β-asp is present in all GF animals studied and that it is 100% degraded in all CV adult mammals studied (Table II). These results indicate that microorganism(s) capable of degrading β-asp are established in all species.

Degradation of β-asp was variable in the fish studied, with a significantly higher degradation in cod than in salmon. As mentioned, fish need a diet with high amounts of proteins (70). The freshwater salmon are farmed with surplus of proteins stimulating their growth rate, ensuring a surplus of β-asp (see Table III). The passage through the GI tract is most likely adjusted to accelerate when intake of feed increases, together with concurrent decreased metabolism of ingesta. The low level of degradation of β-asp in salmon fits in well with these assumptions, together with the fact that fish have relatively few intestinal microbes compared with homeothermic mammals.

Excessive antibiotic treatment of mammals may result in the presence of β-asp (75). As mentioned earlier, the presence of β-asp has been used as a colonization resistance factor (76, 77). In our studies, degradation of β-asp was significantly reduced in the salmon treated with antibiotics compared with the untreated salmon (see Table III). Whether and to what extent the presence of β-asp in fish can be used to express degree of colonization resistance remains to be investigated.

Formation of short-chain fatty acids

SCFAs are intermediates and end products of microbial degradation of exogenously and endogenously derived compounds in the GI tract of all mammalian species. Endogenous production of acetate occurs in the liver and/or in peripheral tissues (33). Faecal SCFAs represent the net sum of production, absorption and possible secretion of SCFAs throughout the GI tract. Two different patterns of SCFA absorption have been claimed to take place in the colon of several mammals as well as in the rumen of sheep (14, 33, 78, 79). Firstly, absorption of the unionized or acid form of SCFAs can occur with luminal accumulation of bicarbonate and an increase in pH. The second pattern involves absorption of the ionized form of the SCFAs, since absorption of sodium occurs at a similar rate to that of SCFAs without appearance of bicarbonate in the lumen (33). Thus, apart from its energy value, absorption of SCFAs must have a significant role to play in sodium and water balance in the body.

The microbial origin of intestinal SCFAs has been substantiated by comparative studies in GF and CV mice and rats (77, 80), and by studies in GF mice monoassociated with probiotic strains (81). In GF animals SCFAs are mainly represented by acetic acid (Table IV), while the other acids are derived from the diet (82). In CV omnivores the proportion of faecal acetic acid is around 50–60%. In herbivores, the total faecal amount of SCFAs was similar in horses and cows, but far less in elks and reindeers. The proportions of acids were similar in horses, cows and elks but significantly different in reindeers. Large differences were observed in the amount and the proportions of SCFAs between the ruminal content and faecal content of cows (Table V).

The major events in ruminant digestion are that complex carbohydrates from the feed undergo microbial fermentation, forming SCFAs that are used by the animal, while methane and carbon dioxide are lost to the atmosphere. In ruminants, the measurable values of SCFAs in the rumen are the most representative for production of SCFAs. The relatively higher faecal concentrations of SCFAs in omnivores than in herbivores probably depend on the fact that omnivores absorb a lower percentage of energy from SCFAs than herbivores. The magnitude of energy support by SCFAs in mammals – 75–80% in cows (33), 25–30% in pigs (16), 5–10% in humans (14, 15) – may partly be reflected in reversed levels of faecal SCFAs. The comparatively high concentration of SCFAs in horses and cows and their low concentration in elks and reindeers is probably because stabled animals have more feed available than wild animals. Similarly, the much lower amounts of SCFAs in reindeers than in elks is probably a consequence of their very sparse diet during the catabolic winter season.

Butyrate is readily oxidized to carbon dioxide in the colonic wall and thus acts as an important respiratory fuel or energy source for the colon, and likewise in the caecum and rumen (33). The lower faecal proportion of this acid in herbivores (Table V) than in omnivores (Table IV) may be

Table III

Four microflora-associated characteristics in faecal samples from salmon bred in freshwater and faecal samples from cod living naturally in sea water[a]

	Salmon		Cod
	Conventional ($n = 39$)	Antibiotic-treated ($n = 20$)	($n = 21$)
Coprostanol (%)	0 (0–26)	0	0
Mucin degradation[b] (%)	66	66	66
FTA (mg/kg)	873 (0–4140)	2547 (36–3564)	1690 (0–3525)
β-aspartylglycine degradation (%)	57 (0–100)	15 (0–100)	100

[a]Values presented are medians, minimum – maximum; coprostanol is given as percentage of cholesterol plus coprostanol; degradation of mucin and of β-aspartylglycine respectively graded according to percentage.
[b]Total degradation of carbohydrates, but no degradation of proteins stained by Coomassie brilliant blue (64).

Table IV

Faecal values of short-chain fatty acids (SCFAs) in germ-free (GF) and conventional (CV) mice, rats and pigs[a]

Omnivores	Total (mmol/kg)	Acetic (%)	Propionic (%)	i-Butyric (%)	Butyric (%)	i-Valeric (%)	Valeric (%)	i-Caproic (%)	Caproic (%)
Caecum fermenters									
Mice GF	15.5	95.8	0	0.5	1	0.8	1	0	1.4
	9.7–19.2	90.9–96.7		0–1.5	0.9–1.3	0–1.1	0.5–1.5	0–2.2	0.7–2.5
Mice CV	112.6	52.2	17.8	1.4	20.6	4.3	4.1	0.7	0.2
	90.6–131.3	43.9–55.3	13.6–20.6	0.6–1.5	18.4–27.6	2.7–5.5	1.7–4.6	0.3–0.8	0.2–0.2
Rats GF	12.3	97.3	0	0.3	0.4	0.2	0	0	0.7
	5.3–14.2	92–99.5		0–7.5	0–2.4	0–1.2	0–1.1		0.5–2.2
Rats CV	67.8	53.8	15.8	1.2	25.7	1.4	1.9	0	0
	47.3–90	49.5–57.9	13.1–19.5	1.1–1.7	17.8–32.8	1.3–1.9	1.7–2.1		
Colon fermenters									
Pigs GF	11.1	97.6	0	0	1.4	0	0	0	1.1
	8–16.1	87.1–100		0–0.5	0–3.8	0–1.5	0–1.6	0–2.2	0–2.9
Pigs CV	118.3	55.5	25.1	1.3	14.0	1.2	1.9	0	0
	88–199.5	50.8–62.8	19–29.7	1–2.1	11.3–18.2	0.9–2.2	1–3.7		0–3.7
Humans	83.5	57.9	16.4	2	17.4	2.7	2.3	0	0.4
	24.2–242.6	33–71.6	7.9–35.5	0.2–4.8	7.7–30.8	0.1–7.9	0–5.3		0–4.5

[a] Values presented are medians, minimum – maximum.

Table V

Short-chain fatty acids (SCFAs) in herbivores[a]

Herbivores	Total (mmol/kg)	Acetic (%)	Propionic (%)	i-Butyric (%)	Butyric (%)	i-Valeric (%)	Valeric (%)	i-Caproic (%)	Caproic (%)
Colon fermenter									
Horse faeces	45.1	72.8	18.2	1.4	4.2	1.1	0	0	0
	11.0–90.5	58.4–86.4	8.8–36.8	0.4–2.7	2.4–9.4	0–3.3	0–1.4		
Rumen fermenters									
Cow faeces	46.1	78.8	13.4	1.4	4.6	0.9	0.7	0	0
	22.1–64.1	71.6–82.5	12.1–17.8	1.0–3.6	3.8–6.6	0–1.5	0–1.3		
Cow rumen	123.1	63.5	16.4	0.8	16.6	0.8	1.2	0	0.5
	80.0–237.1	56.2–71.4	12.2–19.8	0.5–1.1	13.2–20.6	0.5–1.1	0.8–1.9		0–0.7
Elk faeces	24.7	71.3	16.5	3.2	7.9	0.8	0.8	0	0
	21.0–42.1	59.2–81.1	10.9–24.4	0.5–4.2	5.0–12.2	0.8–1.4	0.7–0.9		0–0.4
Reindeer faeces	17.9	89.9	5.5	0.5	1.4	0.7	0.3	0	0
	8.6–53.0	49.3–99.9	0–3.2	0–3.2	0.1–29.3	0–1.3	0–0.9	0–0.7	0–0.9

[a] Values presented are medians, minimum – maximum.

due to the larger GI tract of herbivores. The high values in mice and rats may also be related to their rapid transit time of ingesta. The obvious higher total faecal amounts of SCFAs in mice than in rats may reflect the most rapid transit time in mice, as seen with coprostanol, urobilins and FTA.

Many physiological and clinical roles, ranging from sodium absorption (83, 84) to cancer pathogenesis (85, 86), are attributed to SCFAs, making them an extremely interesting parameter. The mere fact that intake of antibiotics (87, 88), as well as dietary changes (39, 89–91), may cause alteration in excretion of SCFAs, underlines the importance of studying this function in greater detail. The possible consequences of such an alteration should be analysed according to the scheme outlined in Box 1. Such consequence analysis could actually be done for all intestinal microbial activities.

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