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SYMPOSIUM ON 'NUTRITIONAL IMPLICATIONS OF MICROBIAL ACTION IN THE NON-RUMINAL ALIMENTARY TRACT'

Microbes of nutritional importance in the alimentary tract

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Alimentary tracts evolved as an adaptation enabling the animal to sequester food from other animals, yet allowing motility while continuing feeding or other activities. Microbes have a long history in the alimentary tract, residing in all animals from protozoa to mammals, except for some asteroid echinoderms and a couple of gutless bivalve molluscs receiving food by diffusion through the outer body surface (Reid & Bernard, 1980). The gut is anaerobic, and although about 1% of its microbes are euryoxic the great majority are obligately anaerobic, chiefly bacteria but also protozoa.

The competition model of animal–microbe relationships

The carnivorous animal and the food it digests are essentially similar in chemical composition to the body and food of the ubiquitous chemo-organotrophic microbe. Each is potentially food for the other, and in this sense host and microbe are competitors for the same food. Immunological and other adaptations delay the ultimate microbial consumption of animals and prevent invasion of animal tissues by microbes in the alimentary tract, but cannot protect against microbial attack on the foods ingested by the host. This is accomplished by secretion of microbicidal concentrations of acid before action of the host's digestive enzymes.

Flow of the acid-sterilized digesta along the intestine prevents massive microbial invasion from the large intestine, and microbial numbers in the small intestine are so low that the animal is able to absorb essentially all of its enzymic digestion products. Slower passage in the large intestine, coupled with rapid growth results in a massive microbial population in the hind-gut.

The competition host–microbe model is exemplified in various mammals, including the monotremes (platypus and Echidna), carnivores, Insectivora (moles and shrews), Pinnepedia (seals, etc.), most Cetacea (whales), Chiroptera (bats), ant-

Table 1. *Why carbohydrates?*

$4\ HCOOH \rightarrow 3\ CO_2 + CH_4 + 2\ H_2O$ 184 daltons	$\Delta G^{\circ\prime} = -120$ kJ 0·65 kJ/dalton
$4\ CH_3OH \rightarrow 3\ CH_4 + CO_2 + 2\ H_2O$ 128 daltons	$\Delta G^{\circ\prime} = -311$ kJ 2·43 kJ/dalton
$2\ CH_2O \rightarrow CH_4 + CO_2$ 60 daltons	$\Delta G^{\circ\prime} = -176$ kJ 2·93 kJ/dalton
$n\ CH_2O \rightarrow (CH_2O)_n$ Soluble → insoluble, no concentration effect	

and termite-eaters (pangolin, aardvark and spiny ant-eater) and most primates. Birds, reptiles, amphibia and fishes, as well as many invertebrate groups, exhibit this model.

The ubiquity of fibre on Earth. A different host–microbe relationship has evolved in many herbivorous mammals, due to the ingestion of relatively large quantities of plant cell wall materials which are resistant to the animal's enzymes but which are digestible by some microbes. The massive amounts of carbohydrates in plants have resulted evolutionarily from the peculiar suitability of carbohydrates to support biochemical work under anaerobic conditions (Hungate, 1955). Primitively, Earth was anaerobic, with atoms joined randomly into all possible chemical combinations. Oxygen was insufficient to convert all elements into their most oxidized form, and carbon was at intermediate oxidation states. These states could provide metabolic energy anaerobically by conversion to the more stable configurations of carbon dioxide and methane. Of the three intermediate states of CO_2, conversion of formaldehyde to CO_2 and CH_4 could accomplish more biochemical work per unit of weight than could anaerobic conversion of formic acid or methanol (Table 1).

Not only could carbohydrates provide energy but they existed in the form of various complex sugars that, using the energy inherent in their oxidation state, could with appropriate enzyme catalysis rearrange into precursors of amino acids. Further, the sugars could polymerize into insoluble reserves exerting negligible osmotic effects, and also into structural polymers such as cellulose. Primitively, cellulose in the walls of aquatic plants may have prevented plasmoptysis when the protoplast became surrounded by a semi-permeable membrane. After the evolution of photosynthesis using water as the hydrogen donor, carbohydrate became as cheap as air, water and light, and structural cellulose became important and abundant in land plants.

The co-operation model. The abundance of carbohydrate in plant cell walls is the basis for the evolution of the co-operative model for animal–microbe relationships. The carbohydrate polymers of the wall, indigestible by most animals, are digested and fermented by the microbial partner, with the waste fermentation products and the microbial bodies used by the host. Some insects and molluscs digest plant cell walls without the assistance of microbial partners.

The most widely known and economically important example of a co-operative mammal–microbe relationship has evolved in ruminants. The capacious, never-emptying rumen of these animals retards passage of digesta, giving the time needed for solubilization of a major part of the microbe-digestible components of the fibre. Since microbial relationships in ruminants are not the main subject of this symposium, I will not discuss them except to point out that rumen microbes have received more study than those in other herbivorous mammals and, because the nature of their substrates is better known, our understanding of the diverse roles of the various species is greater than for non-ruminants, though still far from complete.

One problem in the co-operation model is the sacrifice to the micro-organisms of the protein which could be digested by the animal's own enzymes. After deamination the C skeletons of the amino acids can supply energy anaerobically, but it is less than that in carbohydrates because the individual carbons were already oxidized and reduced to some extent in the formation of the amino acids.

The competition–co-operation model. This combination model for mammal–microbe interaction avoids this difficulty. This type occurs in rodents, elephants, rock hyraxes, the horse and its relatives, the dugong and manatee, and rabbits and hares, but is best exemplified in the termites. All these examples, as with the competition model, have the advantage that the animal's enzymes act and the products are absorbed before the microbial fermentation. The difference from the competition model lies in the extent to which anatomical modifications of the host favour longer retention of the digesta, with consequent increased solubilization and fermentation. In both models the microbes act after the host has absorbed the nutrients available through its own enzymes, and in both models there is marked microbial activity in the hind-gut. Some mammals salvage the resulting microbial bodies by coprophagy.

The termite example. The most clear-cut evidence that gut microbes can be of unquestionable nutritional importance to the animal has been found in termites (Cleveland, 1926; Hungate, 1938; Yamin, 1981; Odelson & Breznak, 1983). These insects can consume wood that is only 1% soluble in hot water and contains only 0·03–0·05% N, yet they digest half the wood and assimilate as much as half the total N.

The termite gastrointestinal (GI) tract consists of a fore-, mid- and hind-gut. Ducts from salivary glands secrete amylase-containing fluid, mixing in the fore-gut with the finely-comminuted wood nibbled off by the mandibles. Four pouches at the fore- and mid-gut junction secrete a peritrophic membrane extending through the mid-gut and into the hind-gut. Fore- and hind-guts are lined with a chitinous intima, shed during molting. A peptic enzyme is secreted by the mid-gut. The Malphigian tubules carrying the uric acid and other nitrogenous wastes join the hind-gut at its anterior end. This portion of the hind-gut is quite voluminous and contains the symbiotic flagellate protozoa, the great majority of them highly active, ingesting and digesting the small wood particles.

Wood digestion. The composition of the digesta in the fore-gut contents of termites collected from nature resembles that of pellets rather than that of wood (Table 2). However, if the termites are isolated from each other and kept over a screen to prevent reingestion of pellets, the composition of the fore-gut contents resembles that of the wood consumed. The pellets, passing the gut only once, show a much higher lignin content. These results show that the major part of the digestion occurs in the hind-gut, and that the hind-gut material is consumed by other individuals in the colony. Termites practice trophallaxis, stroking the abdomen of a neighbour to solicit voiding of a moist pellet which is consumed. These pellets contain some of the protozoa and bacteria as well as wood residues, and serve to provide protozoa to individuals losing them when the chitinous intima of the hind-gut is shed during molting. Some protozoa are digested but a few pass to the hind-gut region. Dry pellets are not consumed.

As in ruminants the protozoa obtain their energy by fermentation of the cellulose and hemicellulose, as well as a little of the 'lignin' in wood, and the insect absorbs the acetic acid produced, oxidizing it to obtain the major part of its energy. The preponderance of acid as compared with H_2 or CH_4 in the fermentation products has led Odelson & Breznak (1983) to postulate that part of the acetate is formed from CO_2 and H_2.

Nitrogen economy. Study of the N economy of the termite reveals other aspects of the host–microbe relationship. Acetylene reduction tests suggest N fixation in some termites (Benemann, 1973), but these enzymic indications of potential fixation need confirmation by evidence of increase in total N. N balance studies on termites are difficult because in usual short-term laboratory cultures with small amounts of wood the termite N does not increase (Hungate, 1941); there is no net growth.

Table 2. *Composition of termite food and gut contents (mg/g)*•

	Acid and alkali-soluble hemicellulose	Cellulose	Lignin
Natural colony:			
Fore-gut contents	275	221	263
Pellets in gut	248	201	308
Pellets in colony	292	194	290
Wood near burrow	198	466	163
Sound wood	199	492	157
	Acid-soluble		
Isolated individuals:			
Fore-gut contents	162	387	238
Pellets in gut	182	342	399
Pellets in vial	192	373	400
Wood eaten	152	478	233

•Hungate (1938).

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Table 3. *Changes in two 5-year termite cultures*•

Culture . . .	194	198
Wood:		
Eaten (g)	320	58
Oxidized by fungi (g)	1109	222
Nitrogen (mg)	−369	−97
Soil N (mg)	−272	0
Termite:		
No.	+461	+168
Wt (mg)	+2512	+569
N (mg)	+216	+54
Pellet:		
Wt (g)	+160	+30
N (mg)	+410	+47
Total N (mg)	−16	+4

•Hungate (1944).

In one culture containing a large amount of wood and soil a pair of termites produced in 5 years another generation of winged forms (Hungate, 1944). Values for this culture and for a similar one on wood alone are shown in Table 3.

The wood at the termination of both cultures showed obvious signs of fungal attack in the basal portion, particularly in the one with soil, and burrows were more abundant in this region. The weight of wood consumed was calculated from the weight of the pellets on the assumption that half the wood consumed was digested. The wood initially contained less than 0·049% N, yet that assimilated by the insects in the two cultures amounted to approximately one-third and one-half, respectively, of the N in the wood consumed.

This extreme efficiency in assimilation of sparse N is achieved by a combination of factors: (1) any dead or moribund individual in a colony is eaten; (2) fungi assimilate wood N, concentrating it by decomposing 800 times as much carbohydrate, and grow toward the termite burrow; (3) the hind-gut microbiota converts into ammonia the termite nitrogenous wastes entering from the Malpighian tubules (Potrikus & Breznak, 1980). These authors have shown that some of the bacteria in the termite gut can convert uric acid to form acetate, CO_2 and ammonia. The ammonia is assimilated by other bacteria which in turn provide protein for the insect and for the protozoa. Yamin (1981) has pure-cultured some of the termite flagellates, his success being due in part to provision of autoclaved washed rumen bacteria to the cultures, presumably a substitute for the bacteria consumed by the protozoa within the termite.

These interrelationships between the termite and its gut microbiota are reviewed in some detail because they show conclusively that gut micro-organisms can be of very great nutritional importance to the host. In the lower termite families of the *Kalotermitidae* and *Hodotermitidae* the digestion of the carbohydrates of the wood is accomplished chiefly by the flagellate protozoa. They ferment most of the products of wood digestion but it is highly probable that

some carbohydrate leaks from the flagellates and supports fermentation by accompanying microbes. No direct proofs of this have been obtained but the way in which bacteria, and especially spirochetes, so commonly attach to the protozoa suggests a nutritional dependence. *Mixotricha paradoxa*, with its motility supplied chiefly by attached spirochetes, is a striking example of this type of association.

In the *Termitidae*, containing some of the most highly differentiated termite species, the functions performed by the protozoa in the lower families are performed by bacteria. The bacteria comprising the major part of the microbiota in these termites have not been cultured, though some less abundant and presumably unimportant cellulolytic species have been grown (Hungate, 1946).

Mammal–protozoa relationships. Just as isolation has permitted independent evolution of animal species, so have microbiotas evolved in their alimentary tracts. This is best documented in the case of the protozoa because their distinctive morphologies permit more rapid and easy identification of species than is the case with bacteria. Some of the rumen protozoal genera are widely distributed among ruminants, whereas others are quite restricted, one genus being found only in the camel, and another in an antelope. The microfaunas of domesticated cattle and sheep are very similar, probably because of their proximity due to the domestication. Similar genera but different species occur in all individuals of a particular ruminant species, but most individuals in a ruminant herd in a given geographic locality will have similar microfaunas.

Protozoa form an appreciable fraction of the microbial protoplasm in the gut of the animal species listed in Table 4. Many of them have evolved body structures more complex than those in any of their free-living relatives. The hypermastigote flagellates in termites are the most highly differentiated flagellates known, and the ophryoscolecid rumen protozoa similarly include the most complex of known ciliates.

Many of the more highly evolved ciliates are muralytic, as judged by microscopic observation of ingestion of plant cell wall material, and as proven with agnotobiotic clone cultures (Hungate, 1942, 1943; Coleman *et al.* 1976). Many are also amylolytic, and the ciliates in cattle fed on a high-grain ration may become very numerous, and contain as much as 60% of the N in the rumen. Such a large population is capable of digesting the major part of the starch the host consumes.

Table 4. *Incidence of gut protozoa in animals*

Animal	Protozoa	Nutritional importance
Termites	Flagellates	Digest wood, provide nitrogen
Ruminants	Ophryoscolecid ciliates	Muralytic, provide N
Equidae	Cycloposthiid ciliates	Abundant
Hippopotamus	Both types of ciliates	
Quokka	Undescribed ciliates	
Green turtle	Ciliates and flagellates	
Elephant	Polydiniid ciliates	Not numerous
Gorilla	*Troglodytella*, a ciliate	
A few rodents	Ciliates and flagellates	

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Speculations that the cellulases in gut protozoa are of bacterial origin have been numerous. The cellulolytic activity in at least some of the rumen ciliates is not due to ingested cellulolytic bacteria living free within the rumen, but the possibility of cellulase formation by intracellular symbionts has not been excluded. A cellulolytic axenic culture of a termite flagellate showed no evidence of intracellular bacteria when thin sections were examined with the electron microscope (Yamin, 1981).

Bacteria in the rumen are ingested by the protozoa in such large numbers that the direct microscopic bacterial count is reduced to two-thirds or less of that in defaunated controls.

The role played by the gut protozoa in mammals has long been debated. Unless there are distinct superiorities in the protozoal amino acids supplied by the host, interposition of the protozoa in the food chain between the bacteria and the animal would be expected to diminish the net protein of the microbiota. A recent study supports this view (Leng, 1982) and there seems to be no basis for according to the ciliates in mammals the important role played by the flagellates in termites. Defaunated termites survive only 2 or 3 weeks, whereas defaunated ruminants live indefinitely. However, in faunated ruminants the protozoa do supply a part of the nitrogenous food of the host.

The ciliates in non-ruminants have been less-well studied, but there seems to be no reason to assume that their role within the ecosystem differs from that in ruminants. However, their bodies cannot supply protein except through coprophagy.

Gut bacteria. Two axioms of bacterial ecology are (*a*) one cannot change the bacterial numbers or composition of an open natural ecosystem by inoculating with exotic species; (*b*) the numbers and composition can be changed by changing the environment. These are only approximations, and the discovery that the detoxification of the mimosine in *Leucaena* by Hawaiian cattle (Jones, 1981) can be transferred by rumen inoculation to Australian ruminants presents a clear instance that particular bacteria can be missing from an open ecosystem.

Item *b* also needs to be qualified. The numbers and composition of the gut microbiotas can change with time without any discernible change in the temperature, moisture, pH, anaerobiosis, food or other environmental factors. The composition of the microbiota is in a continuous state of flux, with much variation in the detailed characteristics of individual strains of a particular 'species'. Mutants constantly appear, some finding a niche, and displacing other strains and then later being in turn displaced. These changes do not lead to a permanent change in the population, since at various times strains with identical characteristics are found. The changes seem to be fluctuations rather than trends.

These changes may be mediated through viral transfers of genetic information within the population or even through random transfers of genetic information with or without specific intermediaries. But keeping in mind the variabilities in strains likely to be encountered, we can still identify bacterial species consistently isolated in high numbers or with other characteristics that make them of nutritional significance.

The nutritional significance of the bacteria thus depends in part on their available foods. The host consumes and digests carbohydrates, fats and proteins. Of the exogenous food only the host-indigestible carbohydrates reach the hind-gut. The nature of the hind-gut fermentation is determined by the relative amount of exogenous microbe-digestible carbohydrates in fibre as compared with endogenous substrates such as host enzymes, shed epithelial cells and mucins.

Carbohydrate fermentations in open gut ecosystems invariably become acidic. On limited carbohydrate substrate the chief acids produced are acetic, propionic and butyric because the biochemical pathways leading to these products form more ATP and consequently support more growth than when ethanol or lactate is formed. Two factors cause protein fermentations to increase the alkalinity of the system: (a) ammonia is more alkaline than the amino group; (b) less net acid is produced because any acid formed from the acid moiety of the amino acid merely replaces a carboxyl rather than creating one as is the case when carbohydrates are fermented.

In the competition model with minimal fibre entering the animal, the colonic fermentation of the endogenous substrates, chiefly nitrogenous, leads to alkalinity through ammonia and amine production, whereas in the competition–co-operation model the microbe-digestible carbohydrates make fermentation acids relatively more abundant. Visek (1978) has presented evidence that unionized ammonia and amines exert a deleterious effect on animal cells. This suggests a negative aspect of the hind-gut fermentation of nitrogenous materials.

Many lines of evidence indicate that the acidic products of fermentation can be used by animals. This was first inferred by Woehler (1824) when he observed that only the cation of administered acetate was eliminated in the urine. The acetate, propionate and butyrate produced in the colon are absorbed and must be of nutritional significance. Butyric acid increases the rate of division of epithelial cells in the rat colon (Sakata & Engelhardt, 1981), and the volatile fatty acids are used by the gut wall tissues of the pig. Wells & Babish (1980) report that even in monoxenic rats the intestinal tissues show greater development than in axenic controls.

The pH of the gut contents influences the rate of absorption of organic acids and ammonia, since cells are more permeable to the undissociated molecules than to the ionic forms. Acidity favours absorption of the acids, and alkalinity favours absorption of ammonia and the amines.

On the assumptions that fermenters of plant cell wall material in the hind-gut are nutritionally valuable to the host and that bacteria fermenting chiefly protein have an adverse effect, bacteria performing these functions can be regarded as nutritionally important.

Muralytic bacteria. Two genera of muralytic bacteria, *Bacteroides* and *Ruminococcus*, are widely distributed in the alimentary tracts of various mammals (Table 5), and some actively cellulolytic species digest about one-third of the fibre in lucerne (*Medicago sativa*) or grass hay (Hungate, 1966). *Bacteroides succinogenes* occurs in the large intestine of cattle, sheep, humans, several African

Table 5. *Muralytic bacteria isolated from animal gastrointestinal tracts*

Bacterium	Source	Substrates digested
Bacteroides succinogenes	Many guts	Cellulose, plant cell walls
Ruminococcus albus	Many guts	Cellulose, plant cell walls
R. flavefaciens	Rumen	Cellulose, plant cell walls
Butyrivibrio fibrisolvens	Rumen	Many hemicelluloses, starch
Eubacterium cellulosolvens	Rumen, human	Cellulose, hemicellulose, starch
Clostridium lochheadii	Rumen	Cellulose, starch, protein
Micromonospora propionici	Termite	Cellulose, oak wood
Clostridium sp.	Elephant	Cellulose
Bacteroides 0061–1	Human	Hemicelluloses
B. fragilis subsp.	Human	Hemicelluloses
B. vulgatus	Human	Hemicelluloses
Lachnospira multiparus	Rumen	Pectin
Phycomycetous fungi	Rumen	Cellulose, plant cell walls

antelope, the quokka, the rat, langer monkey and the horse. Extended study would probably disclose its presence in many additional alimentary tracts. It adheres closely to digestible fibre and digests the wall material in contact with the cell, forming depressions easily distinguishable under the electron microscope. It is stenotrophic, fermenting only glucose, cellobiose, cellulose and pectin. It liberates muralytic enzymes in small vesicles pinched off from the surface of the cell. Its enzymes appear to cease activity when cell growth has ceased and reducing sugars do not accumulate in spent cultures containing excess substrate. It produces acetic and succinic acid and fixes CO_2 (into succinate).

Both *Ruminococcus albus* and *R. flavefaciens* are also active in digesting cell walls of forage plants and have been isolated from the gut of cattle, sheep, rabbits, humans and the horse. In cellulose agar cultures they produce around the colony a wide zone of clearing in which the cellulose has been digested by extracellular enzymes, and reducing sugars accumulate in spent cultures containing excess cellulose. Cellulolytic strains ferment cellobiose and cellulose and, occasionally, one or two other sugars, including glucose. These species also attach to cellulosic substrates, *R. flavefaciens* as long chains of cocci, but *R. albus* as single or a few cells. *R. albus* produces acetate in culture, whereas *R. flavefaciens* forms chiefly succinate, with some acetate.

Other cellulolytic species encountered in the rumen, including *Butyrivibrio fibrisolvens* and *Eubacterium cellulosolvens,* are digesters of cell walls, the butyrivibrios being quite active on hemicellulosic material. They probably also occur in non-ruminants.

Salyers (Salyers *et al.* 1977; Salyers, 1979) has studied the fermentation of various hemicelluloses by bacteria from human faeces, and shown that many species of *Bacteroides* ferment various mucins as well as components of plant fibre. Nine of the species of *Bacteroides* listed in the Bergey Manual are non-saccharoclastic (peptolytic) as compared with thirteen that ferment at least some sugars.

The genus *Fusobacterium* contains seven peptolytic and nine saccharoclastic species. *Eubacterium* is chiefly saccharoclastic (twenty-six species) with only two that are peptolytic. *E. limosum* occupies a special niche. It ferments methanol, H_2-CO_2, or CO to acetate and butyrate. It ferments sugars exclusively to acetate.

These represent only a small fraction of the genera and species comprising the microflora of the mammalian hind-gut. It is very difficult to single out particular species and assess their nutritional significance to the host. Progress can be made by identifying the natural substrates fermented and preparing them in amounts sufficient to use in culture media for enumeration and identification of the organisms attacking them.

Pure cultures do not necessarily produce the same fermentation products as those formed in the natural habitat. One of the most fruitful approaches to our understanding of the nutritional role of various hind-gut bacteria is the controlled introduction of single and combined species into axenic mice and rats.

We can make some generalizations about the bacteria in open ecosystems: energy-yielding substrate almost universally limits the rate of growth, and acetic, propionic and butyric acids are the chief waste fermentation products. More ATP per carbohydrate molecule can be generated when they are the waste products, and bacteria forming them will be able to grow faster than competitors producing other wastes such as ethanol and lactate (Table 6). This explains the general prevalence of the VFAs in natural ecosystems (Hungate, 1975).

Table 6. *Criteria for possible intermediates in the rumen fermentation*

Intermediate	Concentration (nmol/ml)	μ (/min)	Flux (nmol/ml per min)	Product formed	Rumen production accounted for (%)
Lactate	<12	0·03	0·36	Acetate	<1
Ethanol	Not detected	0·003	—	Acetate	—
Succinate	4	10	40	Propionate	33
Hydrogen	1	710	710	Methane	100
Formate	12	10	126	H_2 and carbon dioxide	18*

μ, specific turnover rate constant for the intermediate pool.

*% of the methane.

This situation is drastically changed if excess easily-fermentable carbohydrate becomes available. At high concentrations of soluble carbohydrate, streptococcal strains can process the sugar molecules faster and can grow more rapidly than can the normal microflora even though obtaining only two molecules of ATP per sugar molecule fermented. The growth energy available per unit time is the key factor in the microbial competition within the ecosystem.

This drastic change in the microbiota occupying the alimentary tract is in marked contrast to the stability of the normal microbiota. The latter is probably more stable in the large intestine of animals exhibiting the competition model, where endogenous substrates predominate, than in the other models where variable proportions of fibre may be consumed.

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