DIGESTION OF MUCIN BY ANAEROBIC BACTERIA OF THE RABBIT CECUM

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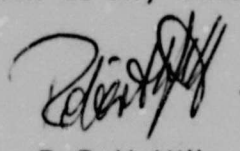
ABSTRACT

The status of mucin as a fermentable energy source for polysaccharide degrading microorganisms in the rabbit cecum was investigated using bacteria isolated from digesta and mucosal membrane. Ultrastructural studies of the cecal epithelium revealed a layer of microvesicles preventing contact between bacteria and the brush border, but there was heavy colonization of the overlying mucous blanket. Mean viable counts from mucus and digesta were 10^6 and 10^9 per ml respectively, with over 18 different species of obligate anaerobes presumptively identified from five rabbits. The species most frequently isolated, identified only on phenotypic criteria, were similar to *Bacteroides vulgatus*, *Streptococcus intermedius*, *Bacteroides ruminicola* and *Peptostreptococcus productus*. There was considerable variation in the distribution of dominant species between mucus and digesta and among individual rabbits. *B. vulgatus*-like strains were not only present in all animals, but were also the most active in degradation of mucin from rabbit small intestines as measured by the periodic acid-Schiff's reaction. Chemical analysis of mucins before and after digestion by *B. vulgatus*-like strains showed that rabbit intestinal mucin was less easily degraded than pig gastric mucin. Nevertheless, there was up to 40 % reduction in carbohydrate content, changes in the relative molar ratios and loss of immuno-reactive terminal structures after digestion by monocultures of mucinolytic bacteria. The glycoside hydrolases likely to be involved in degradation of rabbit mucin were constitutive and cell bound in all strains of *Bacteroides sp.* studied. However, in cultures of mucinolytic strains, lactose, and to some extent mucin, induced higher levels of glycosidases, notably fucosidase and N-acetylglucosaminidase, which were excreted into the extracellular milieu as the cultures approached stationary phase. It is concluded that mucin is a readily available, alternative energy source for polysaccharide-degrading bacteria in the rabbit cecum.

DECLARATION

I hereby declare that the work forming the basis of this thesis is my own and has not been submitted for any degree or examination at any other University



R.R.H.Hill.

DEDICATION

To my Wife,

who lived with an ambition for so long.

PREFACE:

Some of the material presented in this thesis has been published elsewhere.

1. Infection and Immunity, vol.47, pp 540-543, 1985.
   Prevention of adherence by indigenous bacteria to rabbit cecal epithelium by a barrier of microvesicles. R.R.H.Hill.
2. Microecology and Therapy, vol.14, pp 273, 1984.
   Bacterial degradation of cecal mucin. R.R.H.Hill.

List of abbreviations used in the text of this thesis.

GIT: Gastrointestinal tract

VFA: Volatile fatty acids

GLC: Gas-liquid chromatography

SEM: Scanning electron microscopy

TEM: Transmission electron microscopy

PAS: Periodic acid Schiff's reagent

Gal: galactose

Fuc: fucose

GlcNAc: N-acetylglucosamine

GalNAc: N-acetylgalactosamine

For purposes of this thesis, the term "isolate is used for any pure bacterial culture that was grown from a single colony but not identified. The term "strain" is used for a pure culture that was identified in taxonomic studies and allocated a strain number for reference.

ACKNOWLEDGEMENTS

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CHAPTER ONE

INTRODUCTION

1.1 General intestinal microecology

The mammalian gastrointestinal tract (GIT) is an extremely complex system resulting from evolutionary adaption to symbiotic relationships between the host and its colonizing microorganisms. Anatomical divisions of the mammalian GIT show clear differentiation into functional zones such as the oral cavity, stomach, small and large intestines and rectum, each fulfilling a unique digestive process and harbouring its own unique microflora. The many reviews on intestinal microecology of the mammalian GIT have also demonstrated that each part of the system may be subdivided further into different micro-habitats. (Savage, 1978; Freter, 1982). This is well shown from studies of the oral cavity where the saliva, tongue, buccal epithelium and tooth surface support quite different colonizing microflora (Gibbons & van Houte, 1975). Furthermore, throughout the length of the GIT, the colonizing bacteria may be sub-divided into those associated with lumen material, either free, or attached to food particles, those inhabiting the mucous blanket covering the inside of the gut wall and those that adhere to receptor sites on the epithelial surface (Savage, 1978; Costerton & Cheng, 1982). Savage (1977) gives some indication of the diversity of bacterial species inhabiting the GIT of man and there have been many studies on the GIT microbiology of other animals such as rodents, non-human primates, swine, dogs and wild and domesticated ruminants. These reports demonstrate that each species is not only unique in its digestive anatomy, but is also colonized by an original microflora. From the evidence of comparative studies carried out on the microbiology of different

gastrointestinal microhabitats, it is evident that some bacteria occupy highly specific niches, such as the cellulose digesters of the rumen and cecum that adhere to food particles and the spirochetes of primates that are found firmly attached to the epithelial brush border. Alternatively, many of the bacteria that inhabit a particular part of the digestive system may be similar to those that are found in the liquid phase of the lumen. Thus Mead & Jones(1981) reporting on the rumen microflora and Davis et.al., (1977) in a study of bacteria of the dog GIT, recorded similar bacterial genera common to both the epithelial surface and lumen contents, although distributions varied between the two habitats.

The enormous bacterial burdens carried by most parts of the digestive tracts of animals are undoubtedly tolerated because of the benefits gained from symbiotic relationships which outweigh possible disadvantages (McBee, 1971). Studies on germ-free animals reported by Gordon & Pesti (1971), showed that the gut flora performs many essential functions in mammalian digestion, notably, vitamin production, complex polymer digestion, urea recycling, bile deconjugation and mucin regulation among many others. At the same time, the gut epithelium is in a state of mild inflammation with elevated rate of desquamation and there is substantial competition for food resources. Hindgut-fermenting herbivores such as the pig, rabbit and rodents retain digesta for extended periods of time in an enlarged cecal blind sac and some of these animals recycle fecal biomass by obligate coprophagy (McBee, 1977; Williams Smith,1965). In contrast, specialization of the fore-stomach in ruminants permits extensive microbial digestion of plant material and subsequent utilization of the resultant biomass in the gut (Hungate, 1966).

Bacterial population densities in some of the GIT habitats may be as high as 10^{11} per gram of contents in the rumen, for example. These numbers are much reduced in the stomach and proximal small intestine with essentially transient microorganisms in the lumen and sparse autochthonous

populations adherent to the epithelia (Savage, 1983). Towards the ileocecal valve and in the cecum and colon the numbers rise again to between 10^{10} and 10^{11} per gram of contents with large numbers also colonizing the epithelial surface or mucous layer (Moore & Holdeman, 1974; Allison et al.,1979). In those parts of the GIT that have been studied most intensively, there can be many taxonomically distinct bacterial populations, each at densities as high as 10^{8} per gram, but most GIT ecosystems are dominated by comparatively few species that characterize the habitat (Savage, 1977). In general, there are marked differences in the dominant microflora occupying similar parts of the GIT in different animals, but there are also considerable variations among different hosts of the same species and even on a day-to-day basis of any one individual. Moore et al. (1974) showed both qualitative and quantitative differences in human fecal populations between individuals, as did Robinson et al. (1981) in the cecal microflora associated with specific-pathogen-free pigs. As most parts of the GIT of mammals are at very low redox potentials of between -250 to -300 mV, the majority of microorganisms inhabiting the tract are obligate anaerobes, rarely found outside their gut environments. The rumen, colon and cecum ecosystems are the most complex microbiologically, with large consortia of different species of anaerobic bacteria and protozoa contributing to the breakdown of complex plant polymers to yield volatile fatty acids (VFAs) which are absorbed as an energy source by the host (Clark and Bauchop, 1977). Up to 20% of the total energy requirements of hind-gut herbivores is absorbed as VFAs from the cecum with an efficiency equal to that of the rumen (Stevens, 1978; Beauville et al. 1974). There is also a common strategy towards nitrogen conservation among both fore- and hind-gut herbivores in helping to maintain the symbiotic microflora. Urea is recycled through the wall of both the rumen and cecum where it is degraded to ammonia by ureolytic bacteria and released as the main nitrogen source for other gut microorganisms (Hill, 1983; Crociani et al. 1984).

1.2 Anatomy of the cecum

The rumen and its complex microbiology has been the subject of intensive study for many years and is the most fully understood of all the gut ecosystems. More recently, the principles derived from rumen work have been applied to other systems and there are continual additions being made to our appreciation of the role of gut bacteria in man and other animals. The significance of cecal fermentation in hind-gut herbivores of economic importance such as the pig and rabbit, however, is still relatively unexplored. The cecum of the rabbit is a large blind sac terminating in a short appendix. It is thin walled for most of its length, but extensively trabeculated by bands of muscles which keep the organ in continual motion. Contents of the large intestine empty into the cecum at the proximal end near the point of the cecal-colon junction, so that some of the material bypasses the cecum to be excre[illegible] hard feces. There is a diurnal emptying of cecal contents into th[illegible] excretion as soft feces which may be recycled by coprophag[illegible]vens, 1978; Emaldi et al. 1979). The interior wall consists of a simple columnar epithelium and thin musculature, typical of general GIT anatomy. Although the surface lacks villus formation, it is penetrated by numerous indentations termed tubular glands, from which the thin mucous layer originates, secreted by goblet cells lining the gland epithelium (Toner et al. 1971). Material entering the cecum consists of complex dietary polymers that have escaped digestion in the proximal reaches of the GIT together with microorganisms indigenous to these areas. Different species of mucins from saliva, stomach and small intestines also survive the rapid transit from their point of origin to the cecum (Ofasu et al. 1978), where they are supplemented by endogenous cecal mucins (Vercellotti et al. 1978). The cecum, therefore, is an ideal model to study the activities of bacteria that can digest complex polysaccharides from both exogenous and endogenous sources.

1.3 The rabbit cecum as an experimental model

Studies on the microbiology of hind-gut herbivory have concentrated on cecal fermentation of the domestic pig and several reports have shown the pig cecum to be as complex as the rumen, although the microflora consists of bacterial genera more typical of the colon than of those found in the rumen. (Allison et al. 1979; Russell, 1979; Robinson et al. 1984). Furthermore, these studies illustrate the difficulties of general gut microbiology in that they report quite different species structures in different pigs, and even considerable variations between animals in the same study. In comparison to the work done on the microbiology of the pig cecum, however, very little is known about the rabbit, yet this animal is a suitable subject for the study of cecal microbiology as it is also of some importance economically as a source of animal protein. With a protein content of 21%, (higher than that of lean beef), rabbit meat is marketed extensively in Europe, but less so in other parts of the world. The animal is also widely exploited in the fur industry as a souce of coney and angora pelts (Keller, 1965), and is an important subject for animal experimentation in universities and research institutions (Adams, 1976). Yet studies on the digestive process of the rabbit are sparse, and found mainly in European literature.

Early work by Williams Smith (1965) reported that the rabbit cecal microflora consisted almost entirely of *Bacteroides sp.* and low numbers of streptococci. These findings were in accordance with Cools & Jeuniaux (1961), who had reported that the microbial flora of the rabbit cecum was original and included a measure of cellulolytic activity. This theme was explored by several other workers during this period who investigated cellulose degradation in ceca from various animals. Hall, (1952) reported cocci with this property in the rabbit cecum, that were similar to, but not identical with *Ruminococcus sp.* of the rumen. Davies (1965) demonstrated cocci and rods in the rabbit cecum by fluorescent antibody

analysis with antisera prepared against cellulolytic ruminococci and *Bacteroides sp.* isolated from the rumen. Gouet & Fonty, in more general studies (1973, 1979), later described an anaerobic flora of up to 10^9 organisms per gram of digesta which was isolated and enumerated on selective culture media. They reported a mainly gram-negative composition with minor populations of clostridial spore-formers and non-sporulating gram-positive rods. Members of the *Enterobacteriacae* were present at weaning but disappeared from the tract thereafter. Lactobacilli were not recovered from any of the rabbits in their study. This general picture was later confirmed by Christ-Vietor (1973) using similar techniques, but in a subsequent report (Weber et al. 1974) it was established that both lactobacilli and coliforms were present in the GIT of rabbits fed fresh vegatables, but not in those fed a commercial pelleted diet. Two recent reports give some indication of the taxonomy of rabbit cecal bacteria at the species level but both are concerned only with ureolytic strains isolated on selective media. Crociani et al. (1984) identified isolates in the genera *Peptostreptococcus, Fusobacterium* & *Clostridium* as being the main ureolytic strains isolated from cecal contents, although Forsyth & Parker (1985), in a similar study, reported *Bacteroides vulgatus, Clostridium clostridiiforme, Bacillus sp.* and *Staphylococcus sp.* as being the dominant ureolytic bacteria.

The evidence reviewed above suggests that the cecum of the rabbit is a complex ecosystem. Dietary polymers that have survived the host's digestive system, are retained for prolonged attack by microbial populations adapted to this purpose. However, the exact nature of these populations has not been investigated. Comparative studies between conventional animals with a full complement of indigenous microbes, and germ-free animals lacking an intestinal flora, have shown that bacterial fermentation in the cecum degrades 20-30% of dietary polysaccharides before excretion (Gordon & Pesti, 1971). Furthermore, Lindstedt et al. (1965) showed that mucins are excreted almost intact from germ-free rats