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DEGRADATION AND UTILISATION OF MUCINS BY ANAEROBIC

BACTERIA FROM THE COLON

by

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A thesis submitted to the University of Auckland for
the degree of Doctor of Philosophy.

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To my parents for their encouragement and support
of my education which has made this possible.

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ABSTRACT

Intestinal mucins are high-molecular-weight complex glycoproteins which are thought to be fermented by the anaerobic bacteria in the colon. The aim of this thesis was to investigate the mode and extent of mucin degradation by bacteria.

A method was developed to isolate gram quantities of soluble pig colonic mucin and pig gastric mucin. These preparations, along with commercially available pig gastric mucin, were incorporated into bacterial growth media as the sole carbohydrate energy sources. The molecular size and chemical composition of these mucins was determined before and after in vitro digestion by bacteria.

Three different studies were done. In the first, type cultures of *Bacteroides fragilis* were found to be capable of growing on gastric and colonic mucins by cleaving residues from the non-reducing ends of the mucin oligosaccharide chains. Mucin degradation, however, was limited to the removal of only a few residues although this may be significant in vivo as bacteria in the gut lumen must be able to utilise many different complex polysaccharides and thus do not specialise in degrading any one substrate.

In the second experiment, bacteria were isolated from the mucus layer of the pig colon. Three of the cultures studied extensively degraded mucins but colonic mucin was always more resistant to attack than the gastric mucins. Up to 80 mol% of the gastric mucin carbohydrates were cleaved and utilised. In contrast these bacteria utilised no more than 46 mol% of the colonic mucin carbohydrates.

In the third study, fresh rat faecal material was used to inoculate bacterial media containing either colonic mucin or commercial gastric

mucin. The faecal bacteria had completely degraded the gastric mucin after 24 h incubation but 36 h incubation was required to degrade the colonic mucin. However, when bacteria from the colonic mucin tubes were subcultured into fresh colonic mucin it was completely fermented after 24 h incubation.

It is hypothesised that, although the chemical compositions were similar, colonic mucin contained bonds that were not present in the gastric mucin. Evidence for this was obtained by assaying the mucins using the histochemical periodic acid/Schiff and Alcian blue stains. The Alcian blue reactivity was more conserved than PAS staining during bacterial hydrolysis. Therefore hydrolysis of these Alcian blue reactive groups may be the rate-limiting step in colonic mucin degradation.

The molecular basis of the PAS and Alcian blue stains was investigated. PAS stain (0.01% periodic acid) was found to react with terminal neuraminic acid, fucose and possibly galactose residues but not with hexosamine residues. Alcian blue stain bound mainly to sulphate esters but could not be correlated with total sulphate content. When both these colorimetric Alcian blue and PAS stains were used together they provided a valuable tool for assaying the results of chromatography runs. Gastric mucin was shown to be composed of separate neutral (Alcian blue negative) and acidic (Alcian blue positive) glycoproteins. Colonic mucin contained only acidic glycoproteins.

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ABBREVIATIONSCarbohydrates

Fuc	Fucose
Gal	Galactose
GalNAc	N-Acetylgalactosamine
Glc	Glucose
GlcNAc	N-Acetylglucosamine
Man	Mannose
NeuNAc	N-Acetylneuraminic acid
NeuNGc	N-Glycolylneuraminic acid

General

BSA	Bovine serum albumen
CEC	Critical electrolyte concentration
CPC	Cetylpyridinium chloride
DMCC	Direct microscopic clump count
EDTA	Ethy ^{Ethylene} diaminetetraacetic acid
GLC	Gas-liquid chromatography
PAS	Periodic acid/Schiff (stain)
SDS	Sodium dodecyl sulphate
VFA	Volatile fatty acid

DEFINITIONS

The biochemical and histochemical nomenclature of mucus is often ambiguous. It has been recently reviewed by Reid and Clamp (1978) and the following terms have been used in this thesis:

Acidic and neutral mucins	These are used in a histochemical context and denote the staining reaction of the mucins.
Glycoprotein	Protein possessing covalently attached sugars.
Glycosaminoglycan	Carbohydrate portion of connective tissue proteoglycans.
Mucin	Principal glycoprotein component of mucus.
Mucus	Total secretion of the mucous membrane
Sol/gel	The separation resulting from low-speed centrifugation of mucus to give a soluble sol phase and a precipitated gel phase.