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Fermentation of Dietary Starch

In Man

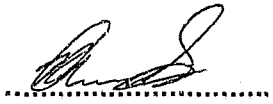
Rashid Ahmed

A dissertation submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Master of Science in Medicine.

Johannesburg, November 1999.

DECLARATION

I, Rashid Ahmed, declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science (Medicine), in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.



Rashid Ahmed

26/11/1999

Date

The work reported in this dissertation was carried out in the Department of Medicine and the Gastroenterology Unit, Chris Hani Baragwanath Hospital, and the University of the Witwatersrand, Johannesburg, South Africa.

This project was approved by the committee for Research on Human Subjects, University of the Witwatersrand.

DEDICATION

To all patients suffering from cancer and alimentary diseases.

In loving memory of my grandparents and late father.

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ABSTRACT

Dietary starch that escapes digestion in the small intestine may be quantitatively more important than dietary fibre as substrate for fermentation. The products of fermentation have important implications in the pathogenesis of colorectal cancer and other diseases of the large bowel which are uncommon in Africans, but have a high prevalence in Western populations.

Maize porridge is a staple of most Blacks in South Africa. Stale maize porridge (high resistant starch – HRS) seems to induce greater fermentation in the large bowel than fresh maize porridge (low resistant starch – LRS).

In the present study, healthy colostomy subjects fed stale maize porridge had significantly more production of SCFA (short chain fatty acids) (mean SCFA – HRS = 182,6; mean SCFA – LRS = 116,1; $p<0,05$) in their colostomy effluent together with a significant drop in stool pH (mean pH – HRS = 5,91; mean pH – LRS = 6,70; $p<0.001$). The SCFA butyrate (mean – HRS = 35,1; mean – LRS – LRS = 17,6; $p<0,05$) and acetate (mean – HRS = 93,9; mean – LRS = 65,8; p

<0,05) were significantly elevated on the stale maize porridge diet when compared with consumption of fresh maize porridge. SCFA, propionate (mean – HRS = 43,1; mean – LRS = 24,8; p=0,05), also increased with stale maize porridge, but was not statistically significant.

A high resistant starch diet and its resultant increase in fermentation products may be partly responsible in protecting the Black population against colorectal cancers and other large bowel diseases.

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CHAPTER 1

INTRODUCTION

Plant fibre components and other nutrients that escape digestion in the small intestine are broken down in the large bowel by anaerobic bacteria by a process known as fermentation. Dietary starch that escape digestion in the small intestine may be quantitatively more important than dietary fibre as substrates for fermentation. The products of fermentation have important implications in the pathogenesis of colorectal cancer and other large bowel diseases.

The incidence of colorectal cancer and other large bowel diseases in South African Blacks is much lower than for Whites. (Walker et al., 1973; Bremner and Ackerman,1970; Segal et al., 1977; Walker and Burkitt,1976). The incidence of colorectal cancers in Soweto was 1/100 000 per year; comparable values for Whites are about 30 times higher (Oettle and Segal, 1988). According to the National Cancer Registry colon cancer was ranked number 7 (in females) and 12 (in males) in South African Blacks compared

to 2 (in females) and 3 (in males) in South African Whites as the most common cancer in 1992 (Sitas et al.,1997).

The observation that people of different cultures have profoundly different disease risks, and that incidences increase with environmental changes, has led to the hypothesis that dietary factors are of paramount importance in colonic carcinogenesis (Wynder and Reddy, 1974; Walker and Burkit, 1976; Waterhouse et al., 1976).

The dietary fibre intake of urban Blacks is very much lower than in Whites and has decreased from 25-30g to 15-20g daily (Bourne et al.,1993). However, the starch intake of urban Blacks are very much higher (212 and 162 g/day compared to 172 and 118 g/day in Black and White males and females respectively) (Vorster,1996). In rural Blacks intake of starch may be even higher. It is suspected that because of limited access to energy sources, many Black households cook maize porridge, the staple and main provider of starch in the diet, not more than once per day. Maize porridge is sometimes eaten reheated or cold, especially so in rural areas. It is suspected that cold maize porridge contains large amounts of resistant starch, much of which will escape digestion in the small bowel and which

will be available for fermentation in the colon. This may explain the striking differences in colon cancer rates.

Resistant starch and other types of starch which escapes digestion in the small intestine may quantitatively be more important as substrates of fermentation than NSP (non-starch polysaccharide). Also, resistant starch is the major substrate for colonic butyrate production. Butyrate is probably the SCFA with the strongest protective effect against colorectal cancers. Furthermore, recent epidemiological data show a negative relationship between starch and colorectal cancer risk (Cassidy et al.,1994).

The patterns of protein and fat intakes of urban Blacks are changing to resemble those of Whites, but are still lower than in Whites (Vorster,1996). These differences could also contribute to the differences in colorectal cancer rates and other large bowel diseases.

CHAPTER 2

LITERATURE REVIEW

2.1 Starches

The starches are polymers of monosaccharide, glucose. They vary in the number of glucose molecules they contain and in their arrangement. Starch granules are composed of linear molecule, amylase, and a branched molecule amylopectin. X-ray crystallography has identified 3 different crystalline structures of the granule: A, B and C. (Katz,1934). Starchy foods such as bread, potatoes, rice, pasta, plantain or cassava form the staple food in most countries of the world. The main sources of starches in the United Kingdom are bread and flour, other cereal products such as rice and pasta, and potatoes. The average daily intake of starches is 138,5 grams which provides approximately 520 Kcal (2216KJ). There is wide individual variation in intakes. A recent national survey of 2000 adult men and women found the median intake of starches for men to be 155 grams per day (range 84-273 grams per day), compared with 106 grams per day for women (range 54-150 grams per day) (Gregory et al.,1990). It is generally assumed that the median starch intake is much higher in developing countries, including most of rural Africa, compared to western countries.

2.1.1 Types of Starches

Until the 1980's it was generally assumed that starch is more or less completely digested and absorbed in the small intestine. Recently, however, studies in men indicated that a considerable amount of dietary starch may escape digestion in the small intestine and pass on to the large bowel where it is readily fermented by microbial flora (Anderson et al.,1981; Stephen et al., 1983; Chapman et al.,1985; Englyst and Cummings, 1985, 86, 87 a). These studies led to a proposed new nutritional classification of starch based on its physical form and susceptibility to pancreatic amylase (Englyst and Cummings, 1987 b).

The main classes of starch proposed are readily digestible starch (R.D.S. 1 and 2), 3 types of partly resistant starch (P.R.S. 1, 2 and 3), and one type (Resistant Starch-RS) totally resistant to digestion in the small intestine of man.

2.1.1.1 Readily-Digestible Starch (RDS) 1

This is dispersed amorphous starch which is highly susceptible to digestion by pancreatic amylase, and includes most freshly-cooked starchy foods. It

is completely hydrolysed in, and absorbed from the small intestine, but may on cooling convert to less digestible types.

2.1.1.2 Readily-Digestible Starch (RDS) 2

This is crystalline starch found in most uncooked cereals and in gelatinised starch dried at high temperature. It is degraded slowly but virtually completely in the small bowel.

2.1.1.3 Partially Resistant Starch (PRS) 1

This includes starch to which digestive enzymes cannot gain access because of physical barriers in the plant tissue e.g. whole or partly milled grains and legumes. Partly digested in the small intestine.

2.1.1.4 Partially Resistant Starch (PRS) 2

This consists of starch granules showing X-ray diffraction pattern type B or C as described by Katz (1937) and is found in raw potato and banana.

2.1.1.5 Partially Resistant Starch (PRS) 3

This consists of mainly retrograded amylopectin. It is formed on cooling moist-heated starchy foods such as potato.

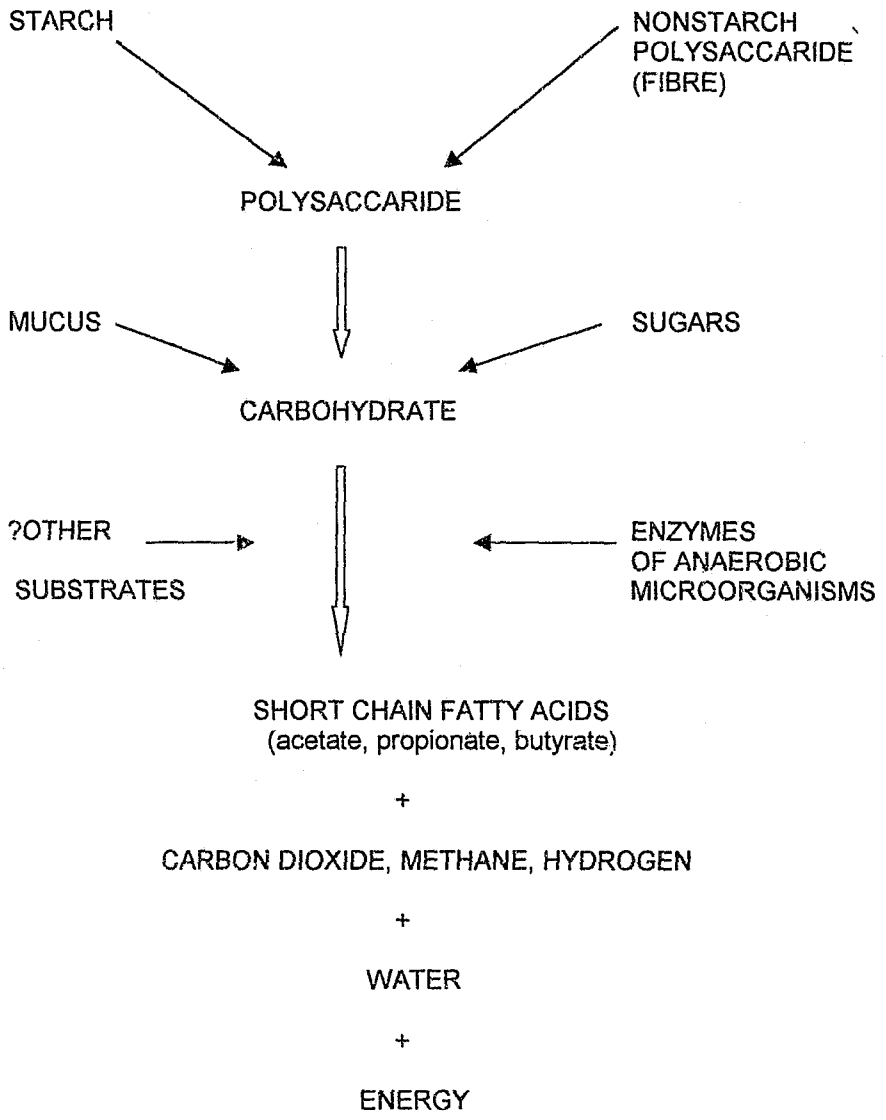
2.1.1.6 Resistant Starch (RS)

Totally escapes digestion in the small intestine and consists mainly of retrograded amylase which is formed on cooling moist-heated starchy foods, for example cornflakes, bread and potato products. The actual amount of RS in food products is small, ranging from less than 1% in bread to 3.1% in cornflakes (Englyst and Cummings, 1987 b) but can be increased to 20% or more during processing. The formation of resistant starch during processing of starchy foods is controlled by water content, pH, heating temperature and time, number of heating cooling cycles, freezing and drying (Englyst and Cummings, 1987 b).

2.2 Colonic Fermentation (See figure 1)

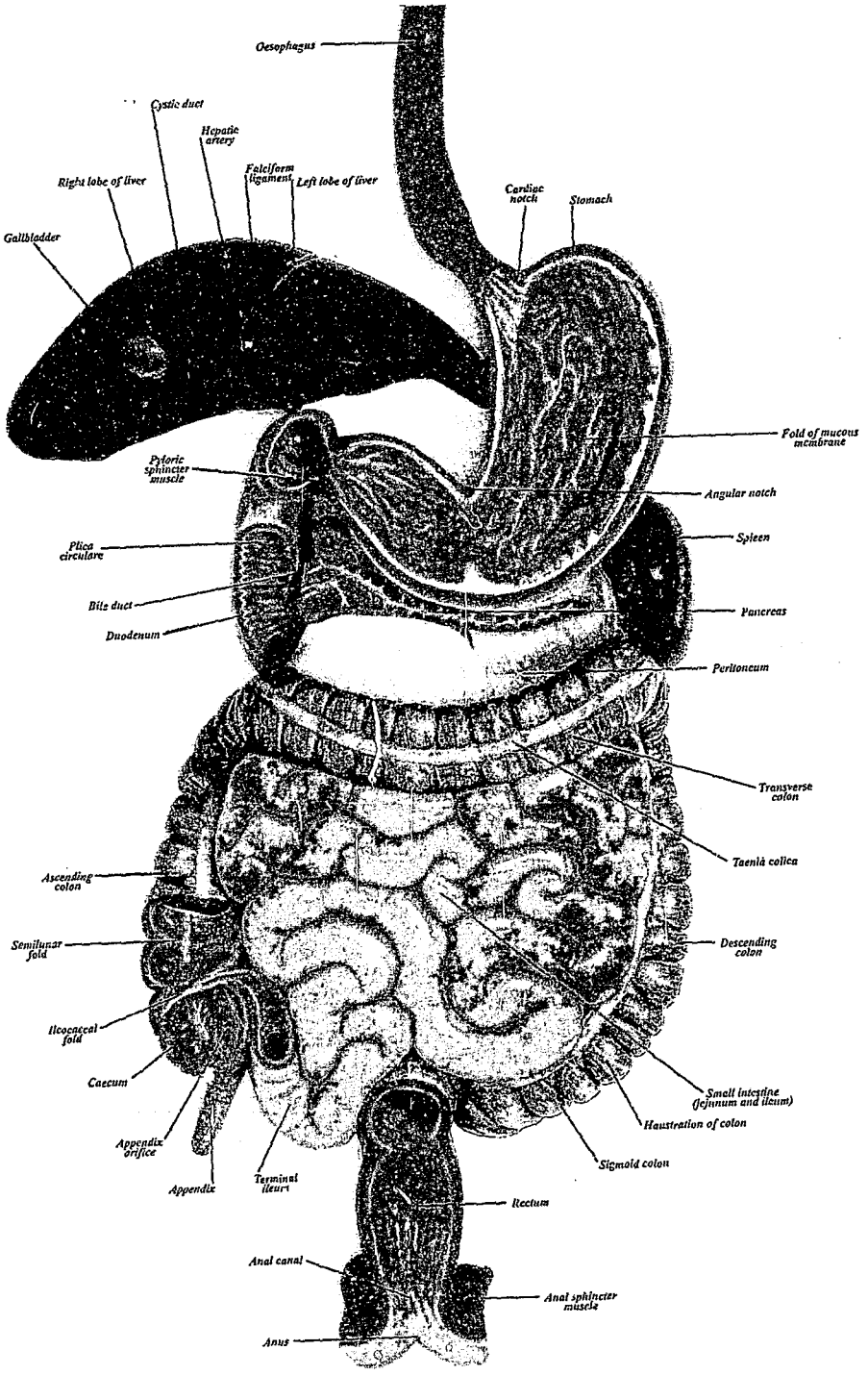
Figure 1

Equation for Fermentation in the Human Colon



2.2.1 Anatomy and Physiology of the Colon and Rectum (See figure 2)

Figure 2.



Human Gastrointestinal Tract

2.2.1.1 Anatomy of the large intestine

The large intestine is about 1,5 m long, extending from the distal end of the ileum to the anus (Williams et al.,1989). It differs in structure, size and arrangement from the small intestine. It has a greater calibre, and a more fixed position. Its longitudinal muscle is concentrated into three longitudinal taenia coli. The colonic wall is puckered into sacculations. Small adipose projections, appendices epiploicae, are scattered over the free surface of the whole colon except over the caecum, vermiform appendix and rectum.

The large intestine curves around the coils of the small intestine, commencing in the right iliac region as a dilated caecum (Williams et al.,1989). The caecum leads to the vermiform appendix and colon. The colon ascends in the right lumbar and hypochondriac regions to the inferior aspect of the liver.

Here it bends (the right colic flexure) to the left with an antero-inferior convexity, loops across the abdomen as the transverse colon to the left hypochondriac region, where it curves again (the left colic flexure) to descend through the left. Here it forms a sinuous loop, the sigmoid colon, continuing along the lower posterior pelvic wall as the rectum and anal

canal (Williams et al., 1989). The caecum lies in the right iliac fossa. It is a large cul-de-sac sac continuous with the ascending colon. Its average axial length is about 6cm and its breadth about 7,5 cm (Williams et al.,1989).

The different parts of the colon will now be discussed briefly (reviewed by Williams et al.,1989).

2.2.1.1.1 The ileocaecal valve

The ileocaecal valve between the small and large intestine, consists of two flaps that project into the lumen of the large intestine. The valve not only prevents reflux from the caecum to the ileum but is probably also a sphincter regulating the passage of ileal contents into the caecum (Williams et al.,1989).

2.2.1.1.2 The ascending colon

The ascending colon is about 15cm long and narrower than the caecum. It ascends to the inferior surface of the right lobe of the liver, where it makes a shallow depression. It turns abruptly forward and to the left at the right

colic flexure. The right colic flexure is at the junction of the ascending and transverse colon. The latter turns down, forward and to the left.

2.2.1.1.3 The transverse colon

The transverse colon is about 50cm long, extending from the right colic flexure in the right lumbar region, across into the left hypochondriac region. Here it curves down and backwards below the spleen as the left colic flexure. The left colic flexure is above and on a more posterior plane than the right flexure at the junction of the transverse colon and descending colon in the left hypochondriac region.

2.2.1.1.4 The descending colon

The descending colon is about 25cm in length, descending through the left hypochondriac and lumbar regions. First it follows the lower part of the lateral border of the left kidney and then descends in the angle between the psoas major and quatratus lumborum to the iliac crest. It then curves downwards and medially in front of the illiacus and psoas major to end in the sigmoid colon.

2.2.1.1.5 The sigmoid colon

The sigmoid colon begins at the pelvic inlet, forming a variable loop of about 40cm and is normally in the lesser pelvis.

2.2.1.1.6 The rectum

The rectum is continuous with the sigmoid colon curving down and back, then downwards and finally down and forward to join the anal canal by passing through the pelvic diaphragm (Williams et al.,1989). The rectum is about 12cm long, with the same diameter as the sigmoid colon, about 4cm in the empty state. The rectum has no sacculations, appendices or mesentery.

2.2.1.2 Histology of the large intestine (See Table 1)

The histology of the colon and rectum is summarized in the following Table:

Table 1

Region	Muscularis externa	Submucosa	Muscularis mucosa	Lamina propria	Surface epithelium
Colon	Outer longitudinal inner circular 3 Bands of taenia	No plicae	Two thin layers	No villi Tubular glands Large lymph nodes Thicker than in small intestine	Simple columnar Few goblet cells.
Rectum	No taenia Much thicker than colon	Isolate lymph nodes Small veins	Not present Lamina propria Submucosa merge here	Thicker than in colon.	Stratified squamous longitudinal folds

Adapted from (Williams et al.,1989)

A major characteristic of the colon is the absence of villi (Martini, 1995). An abundance of goblet cells and intestinal glands are other characteristics. The glands are dominated by goblet cells (Martini, 1995). No enzymes are secreted by the mucosa. Mucus provides lubrication for faecal material when the latter becomes less moist and more compact. Friction or exposure to harsh chemicals stimulate mucous secretions.

2.2.1.3 Physiology of the Large Intestine

2.2.1.3.1 Absorption

The absorptive process in the colon is very important. Approximately 10% of all absorption in the gastro-intestinal tract occurs in the colon (Martini, 1995; Guyton, 1991). Approximately 1,5 litre material enters the colon every day, and only 200ml faeces are excreted. The large intestine (colon) also absorbs, apart from water, other substances such as short chain fatty acids, vitamins, urobilinogen, bile acids, bile salts, and toxins (Martini, 1995). Essentially all ions are absorbed. Ileal effluent contains principally saline solutions with some potassium bicarbonate (Cummings, 1997). The anions found in faeces comprise of short chain fatty acids, bicarbonate, chloride and traces of sulphate and phosphate. Of the cations existing,

potassium, calcium and magnesium with some sodium can be found (Cummings, 1997). Short chain fatty acid transport is a very complex process. Several factors influence this transport by different mechanisms, which is still not yet clear (Van Engelhardt, 1995). Recent studies have shown that a combination of diffusion after protonation and carrier-mediated SCFA anion exchanges with bicarbonate might explain the SCFA transport in the large intestine (Van Engelhardt, 1995).

2.2.1.3.2 The function of the ileocaecal valve

The ileocaecal valve's primary function is to prevent faecal material flowing back into the ileum (Guyton, 1991). The hormone gastrin, secreted from the stomach, increases ileal concentrations and helps relaxing the ileocaecal valve (Guyton, 1991). The prolonged stay of chyme in the ileum facilitates absorption. Strong reflexes from the caecum controls the degree of contraction of the ileocaecal valve, as well as the intensity of peristalsis in the terminal ileum (Guyton, 1991). When the caecum is distended, contraction of the ileocaecal valve is intensified, while ileal peristalsis is inhibited. This delays the emptying of additional chyme from the ileum (Guyton, 1991). The reflexes from the caecum to the ileocaecal valve and

ileum are mediated by the mesenteric plexus in the gut wall itself and also through extrinsic nerves (Guyton, 1991).

2.2.1.3.3 Movements (haustrations) of the large intestine

Distinctive movements of the colon are distinguished, including mixing and mass movements. Circular constrictions combined with longitudinal muscle contractions cause the colon to bulge into sacs called haustrations. This movement provide a small amount of forward propulsion of the colonic material. New contractions occur in other areas nearby, providing a good mixing of faecal material. With this mixing all the faecal material is exposed to the surface of the epithelium.

Propulsion occurs by the analward movement of the haustral contractions and mass movements. Propulsion in the caecum and ascending colon results from slow haustral contractions (Guyton, 1991; Martini, 1995). After this, a part of the distal part of the constrictive ring in the transverse colon, loses its haustrations and contracts as a unit. This forces the faecal

material down the colon until it reaches the rectum (Martini, 1995; Guyton, 1991).

2.2.1.3.4 Defecation

The rectum is usually empty of faeces. When a mass movement forces faeces into the rectum, a desire to defecate is initiated. The rectum contracts and the anal sphincters relax (Martini, 1995; Guyton, 1991).

2.2.2 Substrates for Fermentation

The large gut receives approximately 1,5kg material per day from the small bowel. Plant fibre components and other nutrients that have escaped digestion in the small intestine (dietary component), as well as endogenous carbohydrate and protein (endogenous component), are broken down by anaerobic bacteria in the colon, a process known as fermentation. Most of the fermentation takes place in the caecum and ascending colon.

Although the types of micro-organisms present in the gut will influence SCFA production, the amounts and types of substrates available to the

flora is probably the single most important factor influencing fermentation and the products formed.

Resistant starch and other types of starch which escape digestion in the small intestine may quantitatively be more important as substrates for fermentation than non-starch polysaccharide (Cummings and Englyst, 1987 c). Non-starch polysaccharide form the principle constituents of dietary fibre (Trowell, 1985). Measuring the amount of starch that actually reaches the colon has proved to be extremely difficult. In some studies breath hydrogen excretion has been used as a marker for fermentation (Anderson et al.,1981; Wolever et al.,1986). Other methods used include direct measurements in ileostomy patients. (Chapman et al.,1985; Englyst and Cummings,1985, 86, 87) and intubation of healthy subjects to measure the amount of carbohydrate passing through the ileum (Stephen et al.,1983). Amounts of carbohydrate, malabsorbed from various dietary starch sources, measured by the different techniques, ranged from 2.6% for wheat and potato starch (Chapman et al.,1985) up to 69% from green banana starch (Englyst and Cummings, 1986). From the results of studies reported at that time, Cummings and Branch (1986) concluded that 5-10% (15-20 grams per day) of the starch in western type diets is available for

fermentation. Where starchy foods are the main part of the diet, such as maize in the rural African diet, and especially when it is eaten cooled, much more carbohydrate may escape digestion and absorption in the small intestine. Depending on the type of food and its processing, as much as eight times more starch than non-starch polysaccharide may be available for fermentation (Cummings et al.,1986).

Cummings et al.(1986) and Cummings and Englyst (1987) have tabulated the potential substrates for fermentation.

A. Carbohydrate
    1. Starch
    2. Non-starch polysaccharides (dietary fibre)
    3. Non-absorbed sugars (lactose, lactulose, sorbitol, xylitol)
    4. Oligosaccharides (raffinose and stachyose)
    5. Modified cellulose.

B. Protein
    1. Unabsorbed dietary residues
    2. Endogenous secretions (eg. Pancreatic enzymes).

C. Other substances

1. Mucous (glycoprotein)

2. Dead cells (epithelial and microbial).

2.2.3 The Microflora of the Large Bowel

Non-pathogenic micro-organisms are found in low concentrations in the stomach (gram positive aerobic lactobacilli, streptococci, staphylococci and fungi), in the jejunum (lactobacilli, streptococci and staphylococci) and in the ileum (mainly gram negative bacteria). The colon is the most heavily colonised region of the gut with an estimated 400 different species of bacteria. The dominant species, 99% of the total microflora, in concentrations of $10^8 - 10^{11}$/g, are mainly Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus and Clostridia. Subdominant species include Eschericha coli, Lactobacilli and Streptococci. Although many hundreds of different bacterial species can be isolated from the gut and marked individual variations do occur, most persons within the same racial and socio-economic groups have similar cell population densities and generic distribution of bacteria in their colons.

The gut of babies is inoculated with bacteria from the mother's vaginal and faecal flora during birth. The newborn also acquires bacteria from the environment. The type of feeding also has a strong influence on the microflora and will influence the type of products formed eg. Breast-fed babies produce mainly acetate, bottle-fed babies produce a mixture of SCFA. Weaning will change the microflora to resemble that of an adult at the age of two. In addition to factors such as diet, age and country of residence, a host of other factors will influence the microflora including intestinal anatomy and physiology, host defense mechanisms, colonic transit time, pH, temperature, redox potential and degree of anaerobiosis. The enzymatic potential of intestinal flora is far more versatile and powerful than that of the intestinal secretions. Bacterial enzymes such as amylase, pectinase, cellulose, galactomannase, mucinase, β-glucoronidase, proteases, decarboyxylases, 7 α-dehydroxylase , nitro-reductase and azoreductase ensure that a large variety of substrates are hydrolysed to constituent sugars and amino acids which are further anaerobically fermented to yield energy, the SCFA and several by-products (Macfarlane and Cummings, 1991; Marteau et al.,1993; Roland et al.,1993; Szylit and Andrieux, 1993; Cummings et al.,1995; Macfarlane and Gibson, 1995). Because of the diversity of the microflora, many types of fermentation

occur, mainly classified on the basis of major end products. These include inter alia lactate, alcohol, propionate, acetate and butyrate fermentations. Specialised fermentations such as sulfide and methane fermentation occur because of sulfate-reducing and methanogenic bacteria. The majority of carbohydrate fermenting bacteria in the colon use the Embden-Meyerhoff-Parnas pathway to produce the SCFA and the gases carbon dioxide and hydrogen as the principal end products. The bacteria obtain energy for their cellular function and growth from this process. Intermediates such as lactate and succinate can be further fermented to the SCFA.

Macfarlane and Cummings (1991) pointed out that the types and ratios of SCFA formed by mixed cultures of gut bacteria in vitro, depend largely on the chemical composition of the substrate. Starch fermentation is characterised by high levels of butyrate production while pectin, which is a more oxidised substrate, produces more acetate. Therefore, the products of fermentation are mainly determined by the amount and type of substrate, the rate and extent to which it is broken down, the type of flora and the host factors which influence the flora.

2.2.4 Products of Fermentation

The final products of fermentation are hydrogen, carbon dioxide, methane in a third of the European and North American populations, and short-chain fatty acids (Bond et al.,1971; Pitt et al.,1980; Bjorneklett and Jenssen, 1982). The gases are excreted per rectum or are absorbed, circulated and excreted through the lungs. The short-chain fatty acids in mixed faecal culture include acetate, propionate and butyrate, with acetate as the predominant anion (Rubinstein et al., 1969). Small amount of branched chain fatty acids isobutyrate, valerate and isovalerate are derived from polypeptide fermentation (Thomas et al.,1982; Rasmussen et al.,1988). Other products of fermentation include ammonia, various carbolic and phenolic acids and amines (Cummings and Englyst, 1987). Ammonia may be incorporated into bacterial protein or absorbed depending on the amount of carbohydrate being fermented, whilst the phenols may be excreted in the urine (Cummings and Englyst, 1987). During the process of fermentation materials trapped by fibre, eg. metal ions may be liberated either for use by host or bacteria (Jenkins et al.,1986 a). The short-chain fatty acids produced in the colon lower the pH (Cummings, 1983, Rabblee et al.,1986) and, as a result, 7 α dehydroxylation of bile acids is reduced (Midtredt and Norman, 1968).

2.2.4.1 Short-Chain Fatty Acids (See Table 2)

The term SCFA is usually applied to describe acetic, propionic and n-butyric acids, generated by microbial fermentation in the gut. They were previously referred to as volatile fatty acids. Wrong (1995) defines them as the "saturated unbranched alkyl monocarboxylic acids of 2-4 carbon atoms.

These acids are chiefly responsible for the neutralisation of bases and the acidification of the colon contents and faeces.

Different types of substrates produce different proportion of short-chain fatty acids. All substrates produce acetate as the major end product but the relative amounts of propionate and butyrate vary.

The concentration of SCFA in the colon and faeces is approximately 80-130 mmol/kg. It is highest in the caecum and falls progressively towards the distal colon. The pH is lowest in the right colon (5,6) and rises to 6,3-6,6 in the distal colon. Therefore, it seems that maximal fermentation occurs in the caecum and right colon where carbohydrate substrate availability is the

Table 2

Short chain fatty acids concentrations in human caecum and sigmoid/rectum (Cummings et al.,1987)

	Caecum (mmol/Kg contents (±1 SEM))	**Sigmoid/rectum** (mmol/Kg contents (±1 SEM))
Acetate	69.1 (5.0)	50.1 (16.2)
Propionate	25.3 (3.7)	19.5 (6.7)
Isobutyrate	2.1 (0.4)	1.9 (0.8)
Butyrate	26.1 (3.8)	17.9 (5.6)
Isovalerate	2.7 (0.5)	3.7 (0.9)
Valerate	4.5 (0.5)	4.3 (0.9)
PH	5.6 (0.2)	6.3 (0.2)
No of cases	6	5

greatest. Acetate, propionate and butyrate form 85-95% of the total SCFA with a molar ratio of about 57:22:21 in all regions (Macfarlane and Cummings,1991). The latter may be influenced by the type of carbohydrate in the diet.

Starch yields large amounts of butyrate in an in vitro faecal incubation system whereas xylon and pectin lead to very little butyrate and propionate (Enlgyst et al.,1987 b). In the same system arabinogalactan produces more propionate. Differences in the relative proportions of short-chain fatty acids produced and/or absorbed with different substrates have also been found in vivo in animal studies. In rats, fermentation of guar gum raises propionate concentrations (Tulung et al., 1987); pectin raises acetate and lowers butyrate (Thomson et al.,1984) and gum arabic produces large amounts of butyrate (Topping et al.,1987 b; Tulung et al.,1987). Wheat bran and porridge oats produce mainly acetate and propionate in pigs, with butyrate contributing less than 8% of the total short-chain fatty acids (Topping et al.,1985 a). From the observations of Chang et al. (1987) in rats it appears that the aleuron fraction of wheat bran is responsible for the high yield of propionate. The relative proportion of butyrate is raised by feeding fibre fractions as mixtures rather than as individual components

(Topping et al.,1985 b). When feeding individual components, the short-chain fatty acids profile seems to be related to the monosaccharide composition of the substrate. For example, lactulose is converted to acetate only, as is the case with its monosaccharide components D-galactose and D-fructose (Mortenses et al.,1988). Differences in fermentation profiles from various substrates have also been ascribed to changes in the microflora of the host (Cheng et al.,1987; Tulung et al.,1987).

2.2.4.2 The Fate and Metabolism of the SCFA

The principal short-chain fatty acids produced during fermentation are acetate, butyrate and propionate. Short-chain fatty acids acidify the proximal colon and is important in stool bulking. One molecule of complex carbohydrate yields 10-200 molecules of monosaccharide, each of which yields 2 molecules of short-chain fatty acids. The short-chain fatty acids are rapidly absorbed from the colonic lumen (McNeil et al.,1978; Hoverstad, 1986; Rechkemmer et al., 1988) in a process that stimulates the uptake of sodium and water (Ruppin et al.,1980; Roediger and Moore, 1981).

The SCFA are metabolised to different degrees in the colonic cells. The SCFA disappearing from the lumen do not necessarily reach the blood. Engelhardt (1995) points out that the absorption is non-saturable and concentration dependent, although there may be differences in different parts of the colon and between species.

Butyrate is largely taken up by colonic epithelium, which utilise it to synthesize ketones (Roediger, 1980, 1982). Butyrate also affects nucleic acid metabolism in colonic cells through its capacity to stabilise chromatin structure during cell division (Kruh, 1982; Smith, 1986). Furthermore, butyrate is also known to inhibit tumour growth (Sakata and Yajima, 1984; Nordenberg et al.,1986). Remaining butyrate, propionate and some acetate are cleared by the liver (Cummings et al.,1987). Acetate passes to peripheral tissues where it is metabolised by muscles (Lindeneg et al.,1964; Lundquist et al.,1973; Skutches et al.,1979).

Propionate utilisation by the colonic mucosa is low. Propionate in the portal blood is almost entirely cleared by the liver where it is involved in glucose, glycogen and lipid metabolism. Acetate contributes very little to colonic mucosal metabolism. In contrast to portal vein butyrate and propionate

which are entirely cleared by the liver, only a fraction of acetate (less than 25%) is taken up by the liver. However, this will be more than butyrate and propionate because of the higher concentration of acetate in the portal blood. Therefore, acetate is the SCFA produced by fermentation which reaches the peripheral circulation in appreciable amounts. Here it has a short half life because of rapid metabolism in skeletal and cardiac muscles and brain tissue.

2.2.4.3 Beneficial Effects of SCFA in the Large Bowel

SCFA probably affect bowel motility and habit. NSP affects bowel habit through its chernical and physical characteristics such as water solubility/insolubility, particle size, amount of pentose-containing polymers, viscosity and lignification. Low levels of SCFA stimulate colonic motility while high levels have been shown to inhibit motility. The additional effects of SCFA on microbial growth, resultant increased faecal bulk, water retention and shortened transit times all probably contribute to its beneficial effects on bowel habit.

Butyrate added to cells in culture reversibly arrests cell proliferation, alters cell morphology and ultrastructure and influences gene expression. It is

thought that these effects of butyrate on mucosal epithelial cell growth and integrity are the mechanisms by which butyrate, and therefore diets high in starch and NSP, inhibit tumour growth and protects against colorectal cancer. Butyrate, and to a lesser extent propionate and acetate, induce apoptosis in colorectal tumour cell lines at physiological concentrations (Hague et al.,1995).

Propionate and acetate contribute to the beneficial effects on glucose and lipid metabolism. Propionate stimulates hepatic glycolysis in the fed state and hepatic glucose production in the fasting state. Increased metabolism of glucose, accompanied by lower circulating levels of glucose and insulin, will affect lipid metabolism. Furthermore, propionate may affect lipid metabolism directly by inhibiting fatty acid oxidation. Acetate lowers circulating free fatty acids. Increased blood acetate levels are associated with decreased total and LDL-cholesterol.

2.2.4.4 pH of the Colon

Colonic pH is determined by the relative concentrations of acids and bases. The main bases found in the large bowel are ammonia and bicarbonate. High dietary protein is considered to be a major contributor to the elevation

of colonic and faecal pH. The short-chain fatty acids produced in the colonic lumen lowers the pH.

pH is largely dependant on the balance between ammonia and SCFA. The rate of production and removal of SCFA and ammonia at any site in the colon is in a dynamic state depending on the local availability of substrates, effects of other transient food components and the local microflora. All are constantly changing and vary from subject to subject (Drasar and Hill, 1974). The mean adults faecal pH is about 7, with a range of 5,6-9,4 (Geigy Scientific Tables, 1981; Wrong, 1971).

The change in pH alters the metabolism of bile acids, nitrate, sulphate and is also important in control of absorption and secretion and regulation of colonic cell growth. Epidemiological data suggest that a high faecal pH is associated with increased risk of colon cancer. Thornton (1981) postulated that a high colonic pH promotes colorectal cancer.

Epidemiological data suggest that the increased risk of colonic cancer is correlated with a higher faecal pH (MacDonald et al.,1978; Malhotra,1982; Walker et al.,1986). Although some experimental studies have shown a

protective effect against experimentally induced colonic cancer by acidifying colonic contents (Samuelson et al.,1985), others have shown that a more acidified colonic content is associated with increased cell proliferation (Lupton et al.,1985 and 1988; Jacobs and Lupton, 1986) and enhanced tumorigenesis. It is now clear that simply acidifying colonic contents will not consistently decrease tumorigenesis. Perhaps the key is in how colonic contents are acidified – a decrease in base production or an increase in acid production. Or, more important than colonic pH itself, may be factors affected by pH: the composition and metabolic activity of the microflora, the absorption of a particular luminal constituent, or enzyme activity (Newmark and Lupton, 1990).

2.2.4.5 Breath Hydrogen

Hydrogen is not formed by human tissues and the excretion of hydrogen in the breath and rectally has been used to monitor colonic carbohydrate metabolism in man (Levitt MD, 1969; Bond, Engel & Levitt, 1971; Bond & Levitt, 1976). Hydrogen diffuses into the bloodstream, and excretion in the breath has been extensively used as a marker of oral caecal transit time in humans. Attempts have also been made to quantify the amount of carbohydrate reaching the large gut by comparing breath hydrogen

production with a known amount of fermentable carbohydrate, such as lactulose (Bond & Levitt, 1976). However, this is unlikely to be an accurate procedure, since there are other alternatives for the disposal of hydrogen in fermentation, depending partly on the bacterial flora, including mehtanogenic bacteria (Christl et al.,1989) which themselves are in competition with sulphate-reducing bacteria for hydrogen (Gibson et al.,1988 a). The end product of the activities of sulphate-reducing bacteria, hydrogen sulphide, is highly toxic and may be important in the aetiology of large gut disease (Florin et al., 1989). Only 15% or rural black South Africans at low risk of colon cancer carry suphate-reducing bacteria, compared with 70% of samples from British individuals (Gibson et al.,1988 b). Correspondingly 84% of black African are methane producers compared with 52% of whites (Segal et al.,1988). Methanogenesis appears, therefore, not to be a risk factor for colon cancer, contrary to previous observations (Haines et al.,1977).

CHAPTER 3

AIMS

The aim of the study was to assess the acute effect of high resistant starch diet (stale maize porridge) and low resistant starch diet (freshly prepared maize porridge) on colonic fermentation. The products of colonic fermentation that were studied are the SCFA (acetate, propionate, butyrate) and pH in stools.

CHAPTER 4

METHODS

The protocol was approved by the Ethics Committee of the University of the Witwatersrand.

4.1 Subjects (see fig 3 and 4)

Fourteen healthy male colostomates, all with left-sided colostomies, who gave written consent were studied. All subjects had defunctioning colostomies following t uma. None of the subjects were on antibiotics at least ten weeks prior to the study. All the subjects had their weights taken and were medically examined. All of them were medically normal. None of the subjects had past or present gastrointestinal disease of any significance.

Figure 3.



Colostomy Bags

Figure 4.



A Colostomate

4.2 DIET (see fig 5)

Each subject was given a low fibre diet for the entire duration of the study. The diet was not standardized. The low fibre diet consisted of mainly meat and fish. Each subject was given maize porridge (test meal) according to their weights as follows:

50gm of carbohydrate in 62,7gms of maize

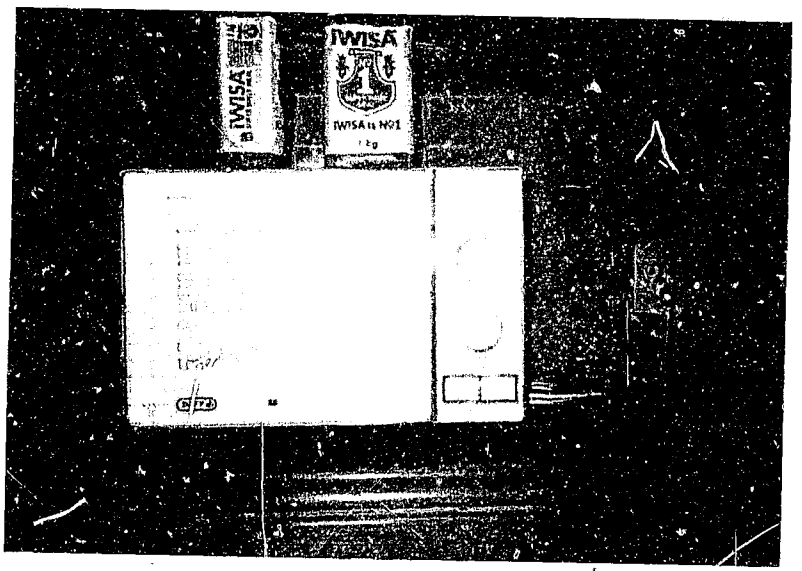
Amount of maize given (in grams) = 62,7 ÷50 x wt. of subject

(i.e. 1gm of carbohydrate per kg of wt)

The maize porridge used was Iwisa No 1 Super Maize Meal, a very refined maize meal with an extraction of about 80%, i.e. most of the fibre is extracted during processing.

The LRS (low resistant starch) diet consisted of freshly prepared maize porridge, cooked in a microwave oven as follows:

Figure 5.



Iwisa No. 1 Super Maize Meal and
the Microwave oven used to cook the meals

Maize weighed out as in the above formula mixed with water equal to 3x wt of maize (ie 1gm of maize = 1ml water). One gram of table salt was added the mixture. The weight of the mixture was taken. The above mixture was then thoroughly stirred and cooked in a microwave oven at maximum power for 3 periods of 2 minutes each and stirred for 1 minute in between. The porridge was then cooled to approximately 40°C. The maize porridge was weighed and any weight loss, as a result of evaporation, was made up by adding water to the maize porridge. The patient then ate the freshly prepared maize porridge without any water.

The HRS diet consisted of stale maize porridge made in exactly the same manner as the LRS diet except for the following:

Water added to the maize was equal to the wt. of the maize and not 3x wt of the maize. The maize porridge was cooled to room temperature and left overnight to be eaten the next day. The subjects ate the stale maize porridge with water equal to 2x the weight of the maize.

The test meal was given to the subjects at 10am every day except on days 4 and 8 when the test meal was given at 8am.

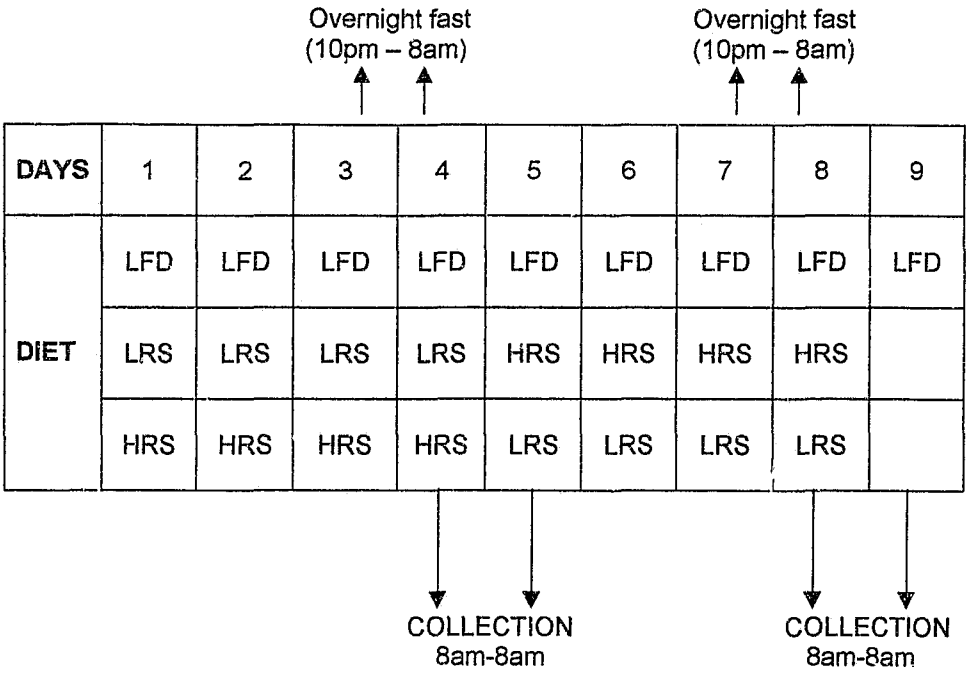
4.3 Test Procedure (see Table 3)

The subjects were given either H/LRS diet on days 1, 2 and 3. They were fasted overnight on day 3. On awakening on day 4 the subjects discarded their colostomy effluent. This was done between 5am and 6am.

The subjects rinsed their mouths with a bacteriacidal mouth wash. At 8am the test meal which the subject had been taking for the last 3 days was given. Colostomy effluent was collected 2 hourly or when available until 8am on day 5.

This was done to avoid colostomy effluent from undergoing fermentation in the colostomy bag. As all the colostomy subjects did not produce stools at regular intervals, the mean pH and SCFA of the stools collected over the 24 hour period was analysed.

Table 3



LFD = low fibre diet
LRS = low resistant starch diet
HRS = high resistant starch diet

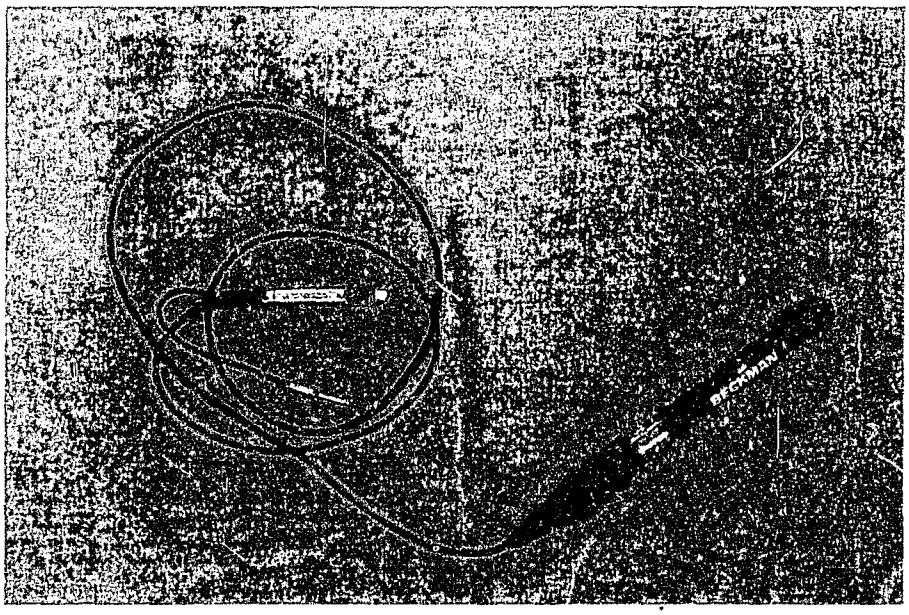
On days 5,6, & 7 the subjects were given the test meal different from the first part of the study, ie subjects who were initially on LRS diet were now given HRS diet and vice versa.

On day 7 subjects fasted overnight. On day 8 the same procedure as day 4 was followed. The subjects were discharged on day 9 after collecting the colostomy effluent at 8am.

4.4 Stool pH (see fig 6, 7 and 8)

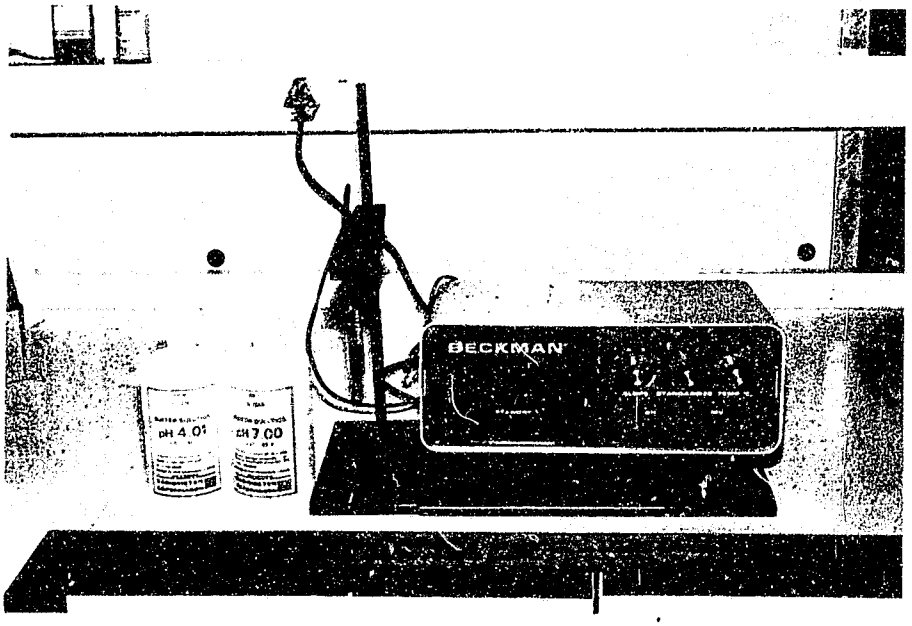
Stool pH was measured using a portable Beckman model 3500 pH digital meter. The apparatus was first calibrated using a buffer solution of pH 7,00 and pH 4,00. One gram of stool was taken from the colostomy effluent which was thoroughly mixed using a wooden spatula. The 1gm of stool was placed in 10mls normal saline and thoroughly mixed using a vortex for 3 minutes. The pH probe was then placed in this stool saline mixture and the pH was read.

Figure 6.



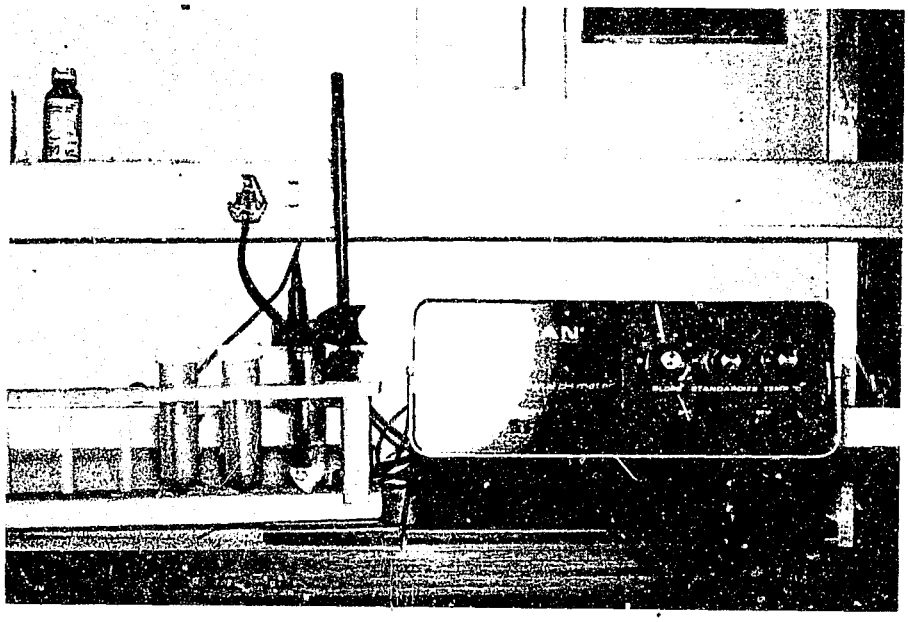
pH Probe – Epoxy body, AgCl, Gel filled Electrode

Figure 7.



Buffers pH 4,0 and pH 7,0

Figure 8.



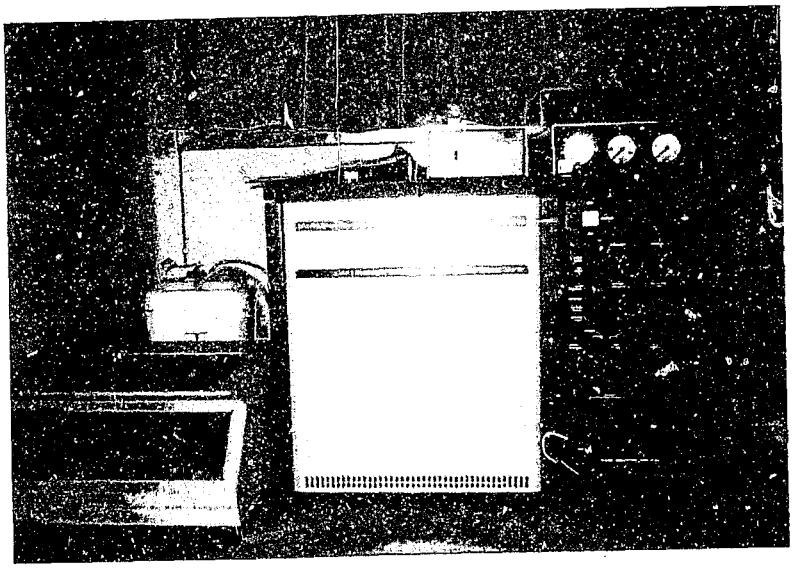
Probe in Emulsion of Faeces with Normal Saline

4.5 S.C.F.A. (see fig 9)

One gram of stool from thoroughly mixed colostomy effluent was placed in a 10mls test tube and stored at -70° for analysis of S.C.F.A. later.

The frozen specimen was thawed at 4°C. The sample was homogenised and diluted with normal saline and centrifuged at 5000 rpm for 15 minutes at 4°C. The supernatant was acidified with sulphuric acid and SCFA extracted in cold ether. 5ul of ether extract with ethyl butyrate as an internal standard was injected into a glass column 6 feet by 2mm packed with Sp 1200/1% H_3PO_3. The injector temperature was 175°C, detector temperature 185°C and column temperature at 125°C. The carrier gas was N_2 at 25mls/mins. The peak area of SCFA peaks was calculated against the areas of the internal standards.

Figure 9.



Phillips Pye Unicam PU-4500, SCFA Detector

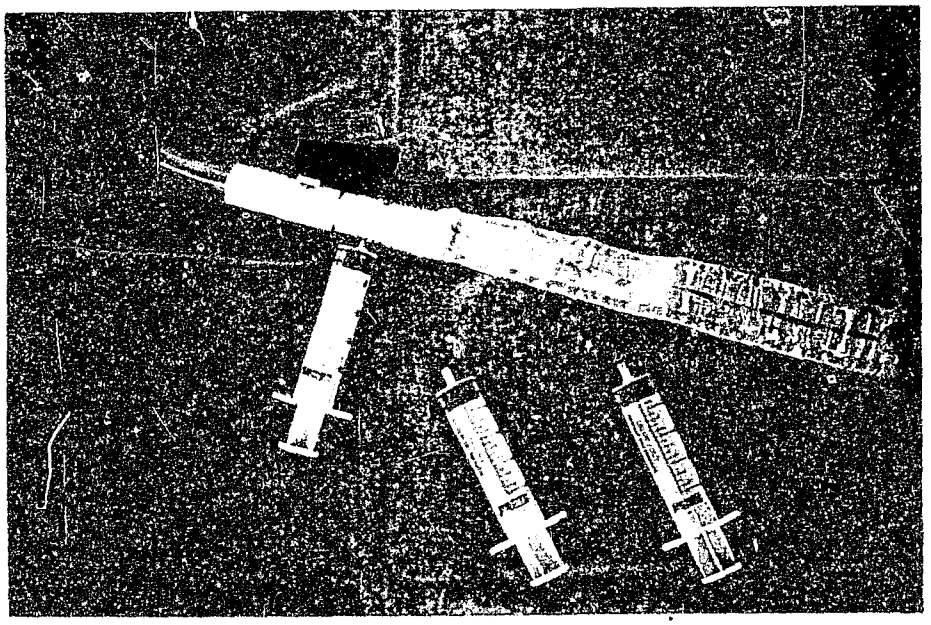
4.6 Oro-Caecal Transit Time (OCTT) (see fig 10 and 11)

Breath hydrogen was measured at 8am (baseline) and then hourly using H.S. Wiggins end expiratory breath sampler into a 20mls plastic syringe. The hydrogen concentrations in the expiratory gas samples was measured using the electrochemical cell – the GMI Exhaled Hydrogen monitor (GMI – Scotland). Calibration of the hydrogen monitor was performed with a standard gas (AGA-gas; AGA, Amsterdam) with a hydrogen concentration of 95,7 ±2,5 ppm.

4.7 Statistical Analysis

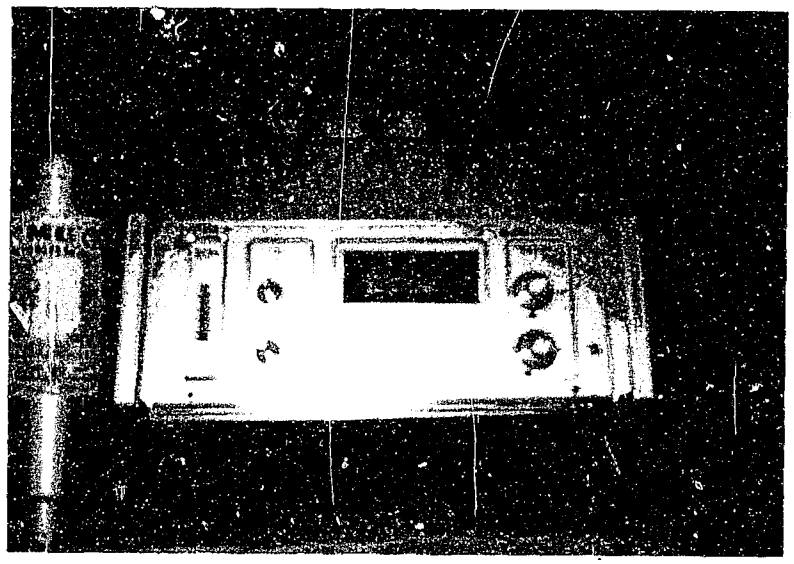
Results were analysed with Statistica Program using the Student T-test for dependent samples.

Figure 10.



H. S. Wiggins End Expiratory Breath Sampler

Figure 11.



GMI Exhaled Hydrogen Monitor
(GMI - Scotland)

CHAPTER 5

RESULTS

(SCFA measured in mmol/kg)

Bold = HRS Diet
Italic = LRS Diet

5.1

Time	pH	Acet	Prop	Isobut	But	Isoval	Val	Cap	Total
				Subject 1					
0hrs	**5,62**								
2 hrs	**5,43**	**69,69**	**27,39**	**0,03**	**18,24**	**0,44**	**2,07**	**0,63**	**118,49**
4 hrs	**5,15**	**84,56**	**36,68**	**0,17**	**23,41**	**0,59**	**2,17**	**1,02**	**148,6**
6 hrs	**6,89**	**48,78**	**15,65**	**0,29**	**10,96**	**1,03**	**0,3**	**1,14**	**78,15**
8 hrs	**5,86**	**63,55**	**29,66**	**0,28**	**20,73**	**0,68**	**2,36**	**1,05**	**118,31**
Mean	**5,79**	**66,65**	**27,35**	**0,19**	**18,34**	**0,68**	**1,72**	**0,96**	**115,89**
0hrs	*7,48*	*50,82*	*17,58*	*0,95*	*11,81*	*1,45*	*3,38*	*0,92*	*86,91*
24hrs	*6,55*	*37,83*	*18,19*	*0,41*	*9,07*	*1,22*	*2,21*	*0,73*	*69,66*
Mean	*7,02*	*44,33*	*17,89*	*0,68*	*10,44*	*1,33*	*2,79*	*0,83*	*78,29*

5.2

				Subject 2					
0 hrs	**5,52**	**20**	**8**	**0,7**	**30**	**1,6**	**2,2**	**0,8**	**63,3**
8 hrs	**7,48**	**38**	**13**	**1,3**	**9**	**2,1**	**2,9**	**0,5**	**66,8**
24 hrs	**6,74**	**90**	**20,75**	**1,12**	**18,6**	**1,67**	**4,92**	**1,75**	**138,81**
Mean	**6,58**	**49,3**	**13,92**	**1,04**	**19,2**	**1,79**	**3,34**	**1,02**	**89,61/3**
0 hrs	*6,70*	*72*	*27*	*2,5*	*38*	*5,0*	*4,7*	*0,5*	*149,7*
2 hrs	*7,58*	*47*	*20*	*1,5*	*15*	*3,0*	*3,0*	*0,5*	*90*
6 hrs	*7,89*	*34*	*17*	*1,9*	*12*	*2,1*	*2,0*	*0,6*	*69,6*
8 hrs	*8,01*	*45*	*17*	*1,4*	*10*	*7,3*	*1,7*	*0,4*	*82,8*
Mean	*7,55*	*49,5*	*20,3*	*1,8*	*18,7*	*4,4*	*2,9*	*0,5*	*98,1*

5.3

Subject 3									
0 hrs	6,48	71,15	28,07	2,11	26,28	3,68	8,03		139,32
2 hrs	6,16	81,56	30,73	2,3	35,19	3,93	9,83		163,54
4 hrs	6,50	27,67	9,37	0,77	8,23	1,32	2,69	1,54	51,59
8 hrs	7,13	48,26	11,37	1,43	7,77	2,96	2,46	1,14	75,39
24 hrs	6,11	61,86	17,78	1,04	13,2	2,02	5,47	4,5	105,87
Mean	6,48	58,10	19,46	1,53	18,13	2,78	5,70	1,44	107,14
0 hrs	*7,58*	*22,27*	*7,78*	*1,22*	*6,51*	*2,07*	*2,56*	*0,89*	*43,3*
2 hrs	*7,76*	*23,44*	*6,57*	*1,95*	*6,96*	*4,34*	*2,95*	*0,63*	*46,84*
4 hrs	*7,68*	*27,52*	*7,58*	*2,2*	*7,81*	*3,67*	*2,22*	*1,89*	*52,89*
8 hrs	*7,87*	*38,08*	*8,06*	*2,56*	*6,9*	*4,31*	*2,58*		*62,49*
Mean	*7,72*	*27,83*	*7,50*	*1,98*	*7,05*	*3,60*	*2,58*	*0,85*	*51,38/9*

5.4

Subject 4									
0 hrs	4,62	76	5	0,4	20	0,3		0,8	102,5
2 hrs	6,49	54	5	0,3	16	0,7		0,3	76,3
24 hrs	4,92	102	8	0,6	56	0,4		0,2	167,2
Mean	5,34	77,3	6	0,4	30,7	0,5		0,4	115,3
0 hrs	*5,13*	*90*	*6*	*0,6*	*17*	*0,6*	*0,1*	*0,2*	*114,5*
2 hrs	*7,38*	*70*	*9*	*0,8*	*22*	*1,2*		*0,3*	*103,3*
24 hrs	*5,56*	*64*	*5*	*0,4*	*20*	*0,5*		*0,2*	*90,1*
Mean	*6,02*	*74,7*	*6,7*	*0,6*	*19,7*	*0,8*		*0,2*	*102,7/6*

5.5

Time	pH	Acet	Prop	Isobut	But	Isoval	Val	Cap	Total
Subject 5									
0 hrs	7,90	20	8	1,9	3,3	3,0	1,8	0,2	38,2
24 hrs	6,79	50	19	2,1	18	3,5	3,8	1,6	98
Mean	7,34	35	13,5	2,0	10,7	3,2	2,8	0,9	68,1
24 hrs	*6,85*	*31*	*18*	*1,2*	*8*	*2,5*	*3,3*	*0,6*	*64,6*
Mean	*6,85*	*31*	*18*	*1,2*	*8*	*2,5*	*3,3*	*0,6*	*64,6*

5.6

Subject 6									
0 hrs	5,83	179	86	10,7	61	7,3	9,5	2,0	355,5
24 hrs	6,59	125	49	4,8	47	12	8,2	1,9	247,9
Mean	6,21	152	67,5	7,7	54	9,6	8,9	2,0	301,7
24 hrs	*7,20*	*74*	*21*	*2,7*	*20*	*4,4*	*4,0*	*0,6*	*126,7*
Mean	*7,20*	*74*	*21*	*2,7*	*20*	*4,4*	*4,0*	*0,6*	*126,7*

5.7

Subject 7									
0 hrs	5,52	73	55	2,4	20	1,3	3,0	0,9	155,6
24 hrs	5,38	98	75	6,2	30	2,0	5,5	1,4	218,1
Mean	5,45	85,5	65	4,3	25	1,6	4,3	1,1	186,8
0 hrs	*6,03*	*73*	*48*	*3,4*	*20*	*1,5*	*3,0*	*0,7*	*149,6*
24 hrs	*6,25*	*90*	*54*	*2,4*	*21*	*2,2*	*4,5*	*0,7*	*174,8*
Mean	*6,14*	*81,5*	*51*	*2,9*	*20,5*	*1,8*	*3,8*	*0,7*	*162,2*

5.8

Subject 8									
0 hrs	**5,27**	**54,7**	**20,3**	**Low**	**78,6**	**0,6**	**2,8**		**157**
8 hrs	**6,38**								
24 hrs	**5,00**	**54,5**	**12,6**	**0,5**	**77,5**	**0,9**	**2,0**		**148**
Mean	**5,65**	**54,6**	**16,5**	**0,3**	**78,1**	**0,7**	**2,4**		**152,6**
0 hrs	*5,60*								
6 hrs	*6,26*	*38,4*	*8,0*	*Low*	*10,5*	*0,4*	*1,7*		*59*
8 hrs	*6,05*	*35*	*5,8*	*1,0*	*12,1*	*1,5*	*1,5*		*56,9*
Mean	*5,97*	*36,7*	*6,9*	*0,5*	*11,3*	*0,9*	*1,6*		*57,9*

5.9

Subject 9									
0 hrs	**4,98**	**123**	**103**	**4,4**	**65,3**	**1,6**	**7,4**	**2,5**	**307,2**
24 hrs	**5,08**	**146**	**91**	**6,4**	**49,3**	**3,5**	**10,6**	**8,6**	**315,4**
Mean	**5,03**	**134,5**	**97**	**5,4**	**57,3**	**2,5**	**9**	**5,6**	**311,3**
0 hrs	*5,12*	*120*	*81*	*2,1*	*50*	*1,0*	*5,3*	*2,3*	*261,7*
24 hrs	*5,39*	*130*	*62*	*4,3*	*34*	*1,9*	*6,8*	*1,5*	*246,5*
Mean	*5,26*	*125*	*71,5*	*3,2*	*42*	*1,4*	*6,1*	*1,9*	*254,1*

5.10

Subject 10									
0 hrs	**5,37**	**260,7**	**100**		**33,1**	**2,6**	**4,3**		**400,7**
24 hrs	**5,22**	**191,3**	**108,3**		**45,7**	**2,6**	**6,8**		**354,7**
Mean	**5,29**	**226**	**104,2**		**39,4**	**2,6**	**5,5**		**377,7**
0 hrs	*7,28*	*145,1*	*38,4*	*1,9*	*21,1*	*5,0*	*5,3*		*216,8*
6 hrs	*7,70*	*83,5*	*17,5*		*8,7*	*3,2*	*2,6*		*115,5*
24 hrs	*7,58*	*101,7*	*25,4*	*2,2*	*23,6*	*4,8*	*4,5*		*162,2*
Mean	*7,52*	*110,1*	*27,1*	*1,4*	*17,8*	*4,3*	*4,1*		*164,8*

5.11

STOOL pH of Subjects 11,12,13 and 14				
TIME	**11**	**12**	**13**	**14**
0 hrs	**5,44**	**5,98**	**6,75**	**5,48**
2 hrs				
4 hrs				**5,93**
6 hrs				
8 hrs				
24 hrs	**6,74**	**5,27**	**5,71**	**5,70**
Mean	**6,09**	**5,63**	**6,23**	**5,70**
0 hrs	*7,17*	*7,01*		*5,70*
2 hrs				
4 hrs				
6 hrs			*6,32*	
8 hrs				
24 hrs	*6,27*	*6,90*		*7,29*
Mean	*6,72*	*6,96*	*6,32*	*6,50*

5.12 **Table 4**

Summary of pH, SCFA and stool weights

(SCFA measured in mmol/kg – dry weight)

ID	PH-H	PH-L	Acet-H	Acet-L	Prop-H	Prop-L	But-H	But-L	SCFA-H	SCFA-L	Stool Wt* H	Stool Wt* L
1	5.79	7.02	66.7	44.3	27.4	17.9	18.3	10.4	115.9	78.3	304	287
2	6.58	7.55	49.3	49.5	13.9	20.3	19.2	18.7	89.6	98.1	242	291
3	6.48	7.72	58.1	27.8	19.5	7.5	18.1	7.1	107.1	51.4	201	198
4	5.34	6.02	77.3	74.7	6	6.7	30.7	19.7	115.3	102.7	185	242
5	7.34	6.85	35	31	13.5	18	10.7	8	68.1	64.6	205	208
6	6.21	7.2	152	74	67.5	21	54	20	301.7	126.7	198	201
7	5.45	6.14	85.5	81.5	65	51	25	20.5	186.8	162.2	207	235
8	5.55	5.97	54.6	36.7	16.5	6.9	78.1	11.3	152.6	57.9	286	275
9	5.03	5.26	134.5	128	97	71.5	57.3	42	311.3	254.1	238	241
10	5.29	7.52	226	110.1	104.2	27.1	39.4	17.8	377.7	164.8	197	256
11	6.09	6.72									269	228
12	5.63	6.96									247	245
13	6.23	6.32									199	209
14	5.7	6.5									265	203

H = HRS diet (stale maize porridge)

L = LRS diet (fresh maize porridge)

Acet = Acetate

Prop = Propionate

But = Butyrate

SCFA = Short chain fatty acids

ID = Subjects

*wt = weight (measured in grams/24hours)

5.13 TabLE 5

Breath Hydrogen Peak (in minutes)

SUBJECT	HRS	LRS
1	120	60
2	–	–
3	–	–
4	60	120
5	120	–
6	–	60
7	–	60
8	–	–
9	180	180
10	–	–
11	–	–
12	180	–
13	180	180
14	–	–

HRS = High resistant starch diet

LRS = Low resistant starch diet

– = no increase in breath hydrogen

5.14 Subjects pH analysis

STATS. BASIC STATS	T-test for Dependent Samples (starch ~ 1.sta) Marked differences are significant at p < .05000							
Variable	**Mean**	**Std.Dv.**	**N**	**Diff.**	**Std.Dv. Diff.**	**t**	**df**	**p**
pH_H pH_L	5.907857 * 6.696429 *	.620498 * .704105 *	14*	-.788571*	.648096*	-4.55266*	13*	0.000542*

5.15 Acetate Analysis

STATS. BASIC STATS	T-test for Dependent Samples (starch ~ 1.sta) Marked differences are significant at p < .05000							
Variable	**Mean**	**Std.Dv.**	**N**	**Diff.**	**Std.Dv. Diff.**	**t**	**df**	**p**
Acet_H Acet_L	93.90000 * 65.76000 *	59.48374 * 34.08167 *	10*	28.14000*	38.61422 *	2.304500 *	9*	0.046655*

5.16 Proprionate Analysis

STATS. BASIC STATS	T-test for Dependent Samples (starch ~ 1.sta) Marked differences are significant at p < .05000							
Variable	**Mean**	**Std.Dv.**	**N**	**Diff.**	**Std.Dv. Diff.**	**t**	**df**	**p**
P H P L	43.05000 24.79000	37.01289 20.94561	10	18.26000	25.83440	2.235128	9	.052257

5.17 Isobutyrate Analysis

STATS. BASIC STATS	T-test for Dependent Samples (starch ~ 1.sta) Marked differences are significant at p < .05000							
Variable	**Mean**	**Std.Dv.**	**N**	**Diff.**	**Std.Dv. Diff.**	**t**	**df**	**P**
Isobut_H Isobut_L	2.280000 1.700000	2.635147 .987702	10	.580000	1.893146	.968822	9	.357947

5.18 Butyrate Analysis

STATS. BASIC STATS	T-test for Dependent Samples (starch ~ 1.sta) Marked differences are significant at p < .05000							
Variable	**Mean**	**Std.Dv.**	**N**	**Diff.**	**Std.Dv. Diff.**	**t**	**df**	**p**
But_H But_L	35.08000* 17.55000*	21.75192* 10.05046*	10*	17.53000*	19.93902*	2.780213*	9*	.021393*

5.19 Isovalerate Analysis

STATS. BASIC STATS	T-test for Dependent Samples (starch ~ 1.sta) Marked differences are significant at p < .05000							
Variable	**Mean**	**Std.Dv.**	**N**	**Diff.**	**Std.Dv. Diff.**	**t**	**df**	**p**
Isoval_H Isoval_L	2.600000 2.540000	2.639023 1.499778	10	.060000	2.095604	.090540	9	.929841

5.20 Valerate Analysis

STATS. BASIC STATS	T-test for Dependent Samples (starch ~ 1.sta) Marked differences are significant at p < .05000							
Variable	**Mean**	**Std.Dv.**	**N**	**Diff.**	**Std.Dv. Diff.**	**t**	**df**	**p**
Val_H Val_L	4.360000 3.120000	2.957552 1.618504	10	1.240000	1.859630	2.108605	9	.064215

5.21 Caproate Analysis

STATS. BASIC STATS	T-test for Dependent Samples (starch ~ 1.sta) Marked differences are significant at p < .05000							
Variable	**Mean**	**Std.Dv.**	**N**	**Diff.**	**Std.Dv. Diff.**	**t**	**df**	**p**
Cap_H Cap_L	1.675000 .775000	1.649892 .500714	8	.900000	1.195229	2.129789	7	0.70692

5.22 SCFA Analysis

STATS. BASIC STATS	T-test for Dependent Samples (starch ~ 1.sta) Marked differences are significant at p < .05000							
Variable	**Mean**	**Std.Dv.**	**N**	**Diff.**	**Std.Dv. Diff.**	**t**	**df**	**p**
SCFA_H SCFA_L	182.6100 * 116.0800 *	108.6114* 63.0795*	10*	66.53000*	74.00721*	2.842782 *	9*	.019315*

5.23 Breath H_2 Analysis

STAT. BASIC STATS	T-test for Dependant Samples Marked differences are significant at p < .05000							
Variable	Mean	Std.Dv.	N	Diff	Std. Dv Diff.	t	Df	p
HRS_Breath H2	135.0000	57.44563						
LRS_Breath H2	135.0000	57.44563	4	0.00	48.98979	0.00	3	1.000000

5.24 Stool Weight Analysis

STAT. BASIC STATS	T-test for Dependant Samples Marked differences are significant at p < .05000							
Variable	Mean	Std.Dv.	N	Diff	Std. Dv Diff.	t	Df	p
HRS_Stool LRS_Stool	231.7143 237.0714	38.08781 31.63363	14	-5.35714	34.62237	-.578949	13	.572519

5.25 Summary of pH and SCFA Analysis

STATS. BASIC STATS	T-test for Dependent Samples (starch ~ 1.sta) **Marked differences are significant at p < .05000**			
Variable	**Mean**	**Std.Dv.**	**N**	**p**
PH_H	5.907857*	.620498*		
PH_L	6.696429*	.704105*	14*	0.000542*
Acet_H	93.90000*	59.48374*		
Acet_L	65.76000*	34.08167*	10*	0.046655*
Prop_H	43.05000	37.01289		
Prop_L	24.79000	20.94561	10	.052257
But_H	35.08000*	21.75192*	10*	
But_L	17.55000*	10.05046*		.021393*
SCFA_H	182.6100*	108.6114*		
SCFA_L	116.0800*	63.0795*	10*	.019315*

CASE	VALID N	MEAN	MINIMUM	MAXIMUM	STD DEV
PH_H	14	5.9079	5.03000	7.3400	.6205
PH_L	14	6.6964	5.26000	7.7200	.7041
A_H	10	93.9000	35.00000	226.0000	59.4837
A_L	10	65.7600	27.80000	128.0000	34.0817
P_H	10	43.0500	6.00000	104.2000	37.0129
P_L	10	24.7900	6.70000	71.5000	20.9456
IB_H	10	2.2800	0.00000	7.7000	2.6351
IB_L	10	1.7000	.50000	3.2000	.9877
B_H	10	35.0800	10.70000	78.1000	21.7519
B_L	10	17.5500	7.10000	42.0000	10.0505
IV_H	10	2.6000	.50000	9.6000	2.6390
IV_L	10	2.5400	.80000	4.4000	1.4998
V_H	10	4.3600	0.00000	9.0000	2.9576
V_L	10	3.1200	0.00000	6.1000	1.6185
C_H	8	1.6750	.40000	5.6000	1.6499
C_L	8	.7750	.20000	1.9000	.5007
SCFA_H	10	182.6100	68.10000	377.7000	108.6114
SCFA_L	10	116.0800	51.40000	254.1000	63.0795

5.26 Comments on Results

14 subjects, with the exception of one (subject 5), showed a significant decrease in stool pH (p 0,005) on stale maize porridge (HRS diet). 4 subjects did not have SCFA analysis. The remaining 10 subjects, with the exception of one (subject 2) showed a significant increase (p 0.02) in total SCFA on stale maize porridge (HRS diet).

There was a significant increase (p 0.02) in stool butyrate on stale maize porridge (HRS diet). Subjects showed a significant increase ($p < 0.05$) in stool acetate levels on stale maize porridge except subject 2, who showed a marginal decrease (49,3 on HRS diet and 49,5 on LRS diet).
There was a trend towards significance with regard to the stool propionate (p 0,052257). The results indicate that stale porridge could have more HRS than fresh porridge.

The stool weights on the two test meals were comparable (Table 2). Mean stool weights on HRS diet was 232 gm and on LRS diet it was 237gm (p value = 0,57). Breath hydrogen was used to measure orocaecal transit time. There appeared to be no difference in the transit times on the two test meals (p value = 1 – paired t test).

CHAPTER 6

DISCUSSION

From the study it appears that stale maize porridge induce more fermentative process in the large bowel. One can assume that stale maize porridge contains more resistant starch than freshly prepared maize porridge. The quantity of resistant starch in the fresh and stale maize porridge in my study has not been measured. The formation of resistant starch during processing of starchy foods is controlled by:

1) Water content – the less water content the higher the resistant starch content. The stale maize porridge was prepared with one third the water content compared to the freshly prepared maize porridge. However, the difference in water content was made up by subjects taking their stale maize porridge diet with water so as not to affect the osmolality and absorption of the maize porridge.
2) Number of heating and cooling cycles. Cooling starch granules results in retrogradation with resultant insolubility and α-amylase resistance. The stale maize porridge was cooled.
3) pH
4) heating temperature and time

5) Freezing and drying.

(Englyst and Cummings, 1987b)

Stale maize porridge produced a significant drop in stool pH, total SCFA, butyrate and acetate.

The mean pH on stale maize porridge was lower than normal (mean pH of stale maize porridge 5,91 compared to normal mean pH range 6,3-6,6). Also the mean total SCFA, butyrate, acetate and propionate on stale maize porridge was higher than in normal (mean total SCFA on stale maize porridge 183 – normal 130; butyrate 35 on stale maize – normal 21; acetate 94 on stale maize – normal 57; propionate 43 on stale maize – normal 22).

Mean stool pH, total SCFA, butyrate, acetate and propionate on freshly prepared maize porridge are comparable with normal.

Cooking or processing may alter the glycaemic index of foods. With the exception of pasta and potato crisps, processed foods produce a higher glycaemic index than conventionally cooked foods, possibly due to an increased degree of starch gelatinisation or increased disruption of the

organized granular structure. The glycaemic index of maize porridge is low and can further be lowered by cooling. This observation is ascribed to the composition of the starch of maize. During cooling, resistant and partially resistant starch are formed from the amylose and amylopectin molecules of maize starch respectively. The glycaemic index of the porridge made of oats or sorghum when compared to maize is lowest for oats and highest for sorghum at 90 and 120 minutes. There is little difference after 180 minutes. This may be of importance in diabetics and obese subjects.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Stale maize porridge (HRS) produces more fermentation than freshly prepared maize porridge (LRS). This may be the reason why Black South Africans are protected against colorectal cancers and other large bowel diseases more prevalent in the Western population. There is international agreement that all Westerners should eat fibre-rich foods, because of its protective effect against many diseases (Walker and Walker, 1985). It is suspected that some of these effects are probably mediated through SCFA production.

The dietary fibre intake of Black South Africans is lower than that of Whites. The patterns of protein and fat intake in Blacks are changing to resemble those of Whites. However, Black South Africans consume more starch than their White counterparts.

Diets rich in resistant starch is highly recommended. Cooked starches should be cooled before they are eaten to increase the resistant starch content.

Resistant starch and other types of starch which escapes digestion in the small intestine may quantitatively be more important as substrates of fermentation than NSP (Cummings and Englyst, 1987). Also, resistant starch is the major substrate for colonic butyrate production. Butyrate is probably the SCFA with the strongest protective effect against colorectal cancers. Furthermore, recent epidemiological data show a negative relationship between starch and colorectal cancer risk (Cassidy et al., 1994).

CHAPTER 8

SUGGESTIONS FOR FURTHER STUDY

To measure resistant starch content in hot and cooled maize porridge and other South African foods.

To measure the effects of hot and cold meals on fermentation stool composition, if any.

How changing dietary patterns of South African Blacks will affect the incidence of colorectal cancers and other large bowel diseases in the future.

The role played by colonic microflora in large bowel disease.

If it is ethically possible, to give a high risk colorectal cancer group of patients a high resistant starch diet over a long period and note any protective effect thereof.

Conduct a similar study in a group of a African patients with large bowel disease (eg colorectal cancer) to see if fermentation is different.

ABBREVIATIONS

A Acet	Acetate
B But	Butyrate
C Cap	Caproate
g gm	grams
H	High resistant starch diet
HRS	High resistant starch
IB Isobut	Isobutyrate
IV Isoval	Isovalerate
kg	kilograms
L	Low resistant starch diet
LFD	Low fibre diet
LRS	Low resistant starch
mls	millilitres
mm	millimetres
mmol/kg	millimoles per kilogram

P	
Prop	Propionate
ppm	ports per million
NSP	Non-starch polysaccharide
RS	Resistant starch
SCFA	Short-chain fatty acids
ul	microlitres
V	
Val	Valerate
wt	weight

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